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SHOOT DEVELOPMENT IN DASYLIRION LEIOPHYLLUM ENGELMANN

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SHOOT DEVELOPMENT IN DASYLIRION LEIOPHYLLUM ENGELMANN

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CHAPTER I

INTRODUCTION

In this dissertation an attempt is made to describe shoot development in Dasylirion Leiophyllum Engelmann from embryo to adult plant. Three aspects of development are considered: (1) structure and development of the shoot apex, (2) initiation and early development of the leaf, and (3) structure and development of the primary and secondary thickening meristems.

Members of the genus Dasylirion are xerophytic fibrous-leaved plants which occur only in North America (Trelease, 1911). Engler (1888) in Engler and Prantl (1889) states that some 40 species occur in Texas and the Mexican highlands. Dasylirion leiophyllum occurs in "southern Texas, in the Rio Grande region, passing into New Mexico and reaching or reappearing in central Chihuahua" (Trelease, 1911).

Newberry (1883) states that the Indians in the region bordering the Rio Grande in Chihuahua and Texas use Dasylirion Texanum for food and also to make an alcoholic beverage called "sotol whiskey." The thick leaf-bases, eaten

raw or roasted, are quite palatable and nutritious.

Engler (1888) in Engler and Prantl (1889) classifies Dasylirion with the order Liliiflorae and family Liliaceae. Hutchinson (1934) places it in the order Agavales and family Agavaceae. Lawrence (1951) states that the "Hutchinson classification is now favored by many authorities as representing phylogenetically a more realistic arrangement."

There have been few researches dealing with developmental changes in the shoot apex during different phases of growth. Some of the more recent studies include those of Ball (1941), Reeve (1948a), and Kasapligil (1951). Ball studied the development of the shoot apex in palms from the embryo to maturity. Reeve traced ontogeny of the shoot apex in Pisum from stages in embryogeny. Kasapligil studied developmental changes in the shoot apex of Umbellularia californica and Laurus nobilis.

In this investigation the organization of the shoot apex will be described in terms of the tunica-corpus concept of Schmidt (1924). With certain exceptions which will be discussed later, his terminology is applicable to the shoot apex of Dasylirion. For brevity, tunica layers will be referred to as T-1, T-2, T-3, etc. According to Foster's (1949) review of the tunica-corpus concept the term "tunica" refers to the layer or layers of the shoot apex in which divisions are predominantly anticlinal with respect to the surface of the apex, except where leaf and axillary bud

initiation occur. The term "corpus" designates that part of the apex in which divisions occur in various planes resulting in growth in volume.

The time interval between the appearance of successive foliar primordia will be referred to as a "plastochron." According to Esau (1953) this term was used by Schmidt in 1924, and "was originally formulated in a rather general sense for a time interval between two successive events that are repeated periodically" (Askenasy, 1880).

In the time interval between the appearance of successive leaf primordia the shoot apex undergoes changes in width. Schmidt (1924) introduced the terms "minimal-area" and "maximal-area" phases to describe these changes. According to Gifford (1954) Schmidt "obviously included the leaf buttress in the determination of size and form of the shoot apex." In Dasylirion it appears that the maximum-area phase is reached just before primordia are initiated. Measurements of width do not include the foliar buttresses.

Much of the early work on leaf initiation in angiosperms in general has been summarized and correlated by Foster (1936). Studies of individual plants have been made on Triticum (Rösler, 1928), Alstroemeria aurantiaca (Priestley, Scott and Gillett, 1935), Avena (Kliem, 1937), Tulipa (Sass, 1940), and Sinocalamus (Hsü, 1944). More extensive studies include: (1) those of Rüdiger (1940) on Tradescantia, Iris, Allium, Aloe, and Vanilla,

(2) Schalscha-Ehrenfeld (1940) on Typha, Potamogeton, and Heteranthera, (3) Sharman (1940) on grasses, (4) Thielke (1951) on Dactylis, Zizania, Bambusa, and Saccharum, and (5) Stant (1952) on Elodea, Convallaria, Carex, Luzula, and Narcissus.

Articles which deal exclusively with problems of primary thickening are rare, and most references to the subject occur as scattered comments among numerous papers which go back to the first half of the past century. A brief perusal of this literature reveals that the differences between primary and secondary growth are by no means always clear. The prevailing distinction is based upon the presence or absence of growth in length. Any thickness which occurs while growth in length is in progress is regarded as primary; any which occurs after growth in length has ceased is regarded as secondary.

Some of the earlier studies on thickening include those of Karsten (1847), Schacht (1852), Von Mohl (1858) Rösler (1889) and Frank (1892).

Peterson (1892-93) made an extensive study of thickening growth in monocotyledons. He concluded that there were two extremes in the monocotyledons: One where no meristem occurs beyond that which constitutes the shoot apex and another in which a meristem causing unlimited growth in thickness is established.

Cambium-like meristems have been reported in a

number of monocotyledons since the time of Peterson's work. Strasburger (1906) reported one in the shoot of Washingtonia filifera. Lindinger (1908) reported one just below the shoot apex of Aloe dichotoma. Hausmann (1908) found one in the upper part of the axis of Nolina recurvata. Carano (1910) stated that in Yucca aloifolia the meristem was near the shoot apex. Schalscha-Ehrenfeld (1940) reported one in Typha angustifolia. Ball (1941) studied the development of the primary thickening meristem in palms. Eckardt (1941) carried out a critical investigation on primary thickening in monocotyledons and its relation to secondary thickening. He states that primary thickening meristems occur in Stratiotes aloides L., Typha latifolia, Sparganium ramosum, Iris germanica, Allium cepa, Kniphofia uvaria, Liriope muscari, and Mondo japonicum.

Secondary meristems have also been reported in a number of monocotyledons. Early workers (Schacht, 1852) (Von Mohl, 1858) were aware of continued cambial activity in such woody forms as Dracaena. Scott and Brebner (1893) reported on the formation of a secondary meristem or cambium with a definite initial layer in Aristea corymbosa (Iridaceae). Baranetsky (1897) stated that a permanent cambial zone was formed in Dracaena eliptica. Strasburger (1906) found what he considered to be secondary growth in older parts of the shoot of Washingtonia filifera. Lindinger (1908) mentions a secondary meristem for Aloe

dichotoma. Carano (1910) believed that one was eventually established in Yucca aloifolia. Schoute (1912) believed that secondary growth occurred in about half of the palms he studied. Chamberlain (1921) described a secondary meristem for Aloe ferox. Skutch (1932) reported that banana has a cambium similar to that of Dracaena, but of much shorter duration. Helm (1936) studied secondary growth in palms. He concluded that cell enlargement alone could not account for thickening of these plants.

Eckardt (1941) points out that secondary growth by means of a "Verdickungsring" occurs in the Liliaceae, Amaryllidaceae, Dioscoreaceae, Taccaceae, Iridaceae, Scitamineae, and Bromeliaceae. It reaches its greatest development however in the tree-like Liliiflorae as Aloe, Yucca, Dracaena, Cordyline, and in certain members of the Amaryllidaceae such as Agave and Fourcroya.

CHAPTER II

GENERAL MORPHOLOGY

According to Benson and Darrow (1944), members of the genus Dasylirion are large plants resembling Yucca or Nolina bigelovii. Young plants typically appear as shown in figure 1. Somewhat older plants can be recognized by the dead leaves which begin to accumulate around the base of the stem. In adult plants (fig. 2) this region of dead leaves is very conspicuous. A very common growth form is that in which two and sometimes three or more plants appear to arise from a single mass of dead leaves (fig. 3).

In the vegetative state of Dasylirion the leaves are the most conspicuous parts. Benson and Darrow (1944) describe these as linear, elongated, and not spine-tipped. They also state that the leaf margins bear conspicuous prickles. A close view of an adult plant (fig. 4) shows that the marginal spines are extremely numerous. According to Hutchinson (1944), the leaves are crowded on the stem, and Coulter (1891-94) states that the caudex bears a dense rosette of leaves. McDougall and Sperry (1951) state that the leaves form a basal cluster, and are about half an inch wide and from one to three feet long. Part of a caudex with

All photographs represent Dasylirion leiophyllum unless otherwise stated.

Figure 1. Habit photograph of a small specimen of Dasylirion leiophyllum. The site is a west sloping rocky hillside near Alpine, Texas. The plant is approximately 16 inches high.

Figure 2. Habit photograph of an adult specimen. The plant is nearly 5 feet high. The site is near Sanderson, Texas. Note the dead leaves, which completely cover the stem.

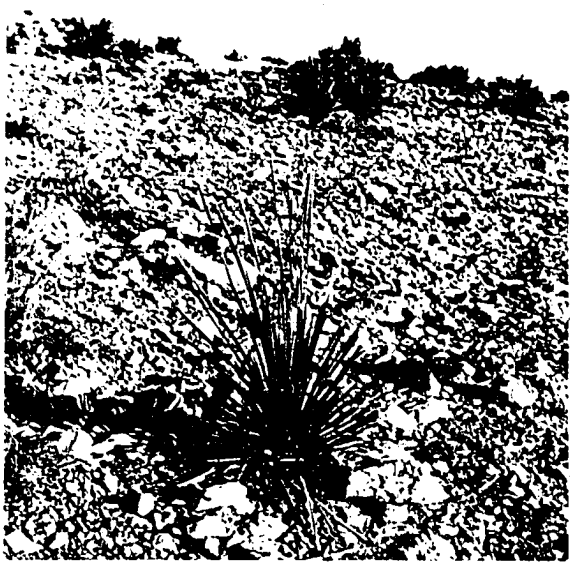
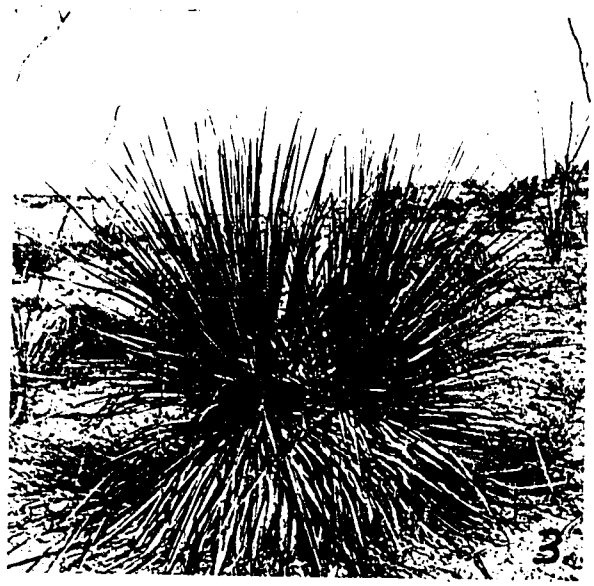


Figure 3. Habit photograph of an adult specimen. The site is near Alpine, Texas. This plant is approximately 4 feet high. The occurrence of two clumps of leaves is due to branching of the axis.

Figure 4. Photograph of a specimen cut lengthwise showing the stem and the attachment of the leaves. This plant is approximately the same size as the one in figure 1. The stem is approximately 1 inch in diameter.

Figure 5. Photograph of part of an adult specimen showing the manner of branching. The part of the stem shown is approximately 4 inches in diameter. Note the rosette of leaves and the expanded basal portions of individual leaves. Spines are visible along the margins of the leaves.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its rosette of leaves is shown in figure 5. The leaves are dilated at their bases and are attached to the stem or caudex as shown in figure 4. When the dead leaves (fig. 2) are removed, the glistening white bases of the living leaves can be seen ensheathing the axis (fig. 5).

As in a large proportion of monocotyledons (Arber, 1925) the axis of Dasylirion is abbreviated. The shape and size vary, depending upon the age of the plant. Seedlings (fig. 6) and very young plants (fig. 7) have an axis that is more or less ellipsoidal (fig. 8a). In an older plant (fig. 1) the axis is often conspicuously ellipsoidal (fig. 8b). However, in plants of the size shown in figure 1, and in somewhat larger ones, it shows other characteristics (fig. 8c, d, e, f). In very large plants (fig. 2), which have a large zone of dead leaves, the axis is usually elongated. Figure 9 shows a large plant with the dead leaves removed. The axis is wider at the base than at the tip.

According to Arber (1925), the secondary thickening of monocotyledons tends to begin late, often lagging far behind the growth in length of the axis. The result is that the stems are sometimes quite massive at the base, but taper rapidly above.

As mentioned previously, plants in which two, three, or more heads appear to arise from a common zone of dead leaves are often found (fig. 3). When the dead leaves are removed from such specimens (fig. 3), it becomes apparent

Figure 6. Photograph of seedlings a-d are 15 weeks old, while e-g are 42 weeks old. The longest leaves on the 42-weeks' old seedlings are about 10 inches. Note the variation in the sizes of the 15-weeks' old seedlings. The root on "a" is the primary root; the roots on all the others are adventitious.

Figure 7. Photograph of young plants, collected near Alpine, Texas. Some of the outer leaves have been removed. The plant in "a" is 7 inches high and 7 mm in diameter at its base. It is devoid of spines except for a few at the base of the leaves. The leaves of the other specimens have spines, and, except for size, resemble the plant shown in figure 1.

Figure 8. Photograph of stems of young and medium-sized plants. The rounded or somewhat flattened top of the stems is the region where the living leaves were attached. The stem at "a" is 17 mm in diameter and 19 mm high; "b" is 35 mm in dia. and 29 mm high. The one at "f" is 35 mm in diameter at its widest point and 55 mm high. The stem at "a" is from the plant in figure 7f. Note the adventitious roots on "b". The projection at lower right on "f" represents the very early stem.

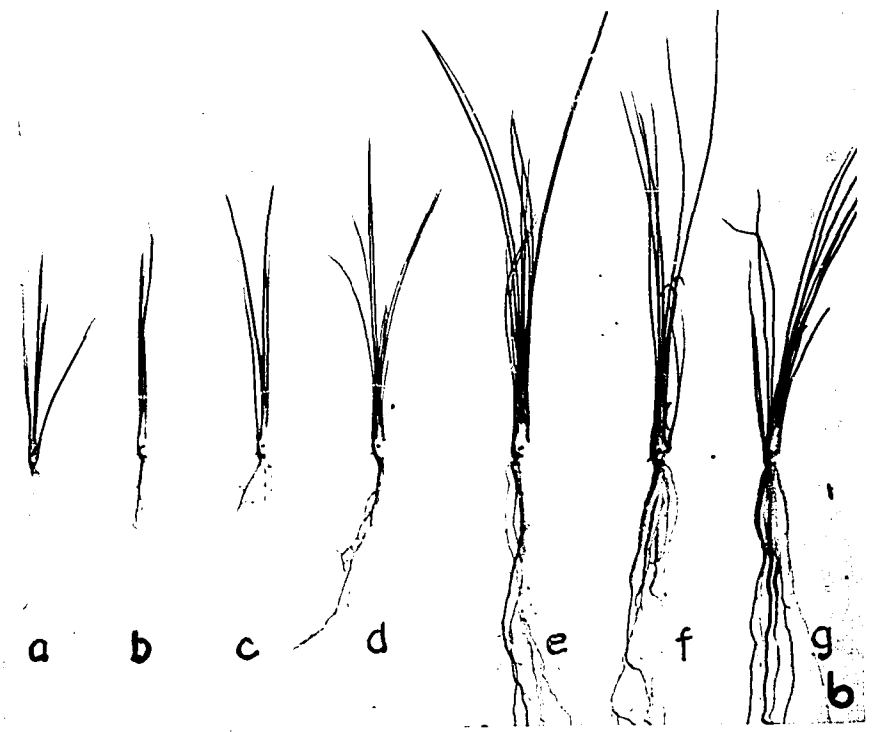

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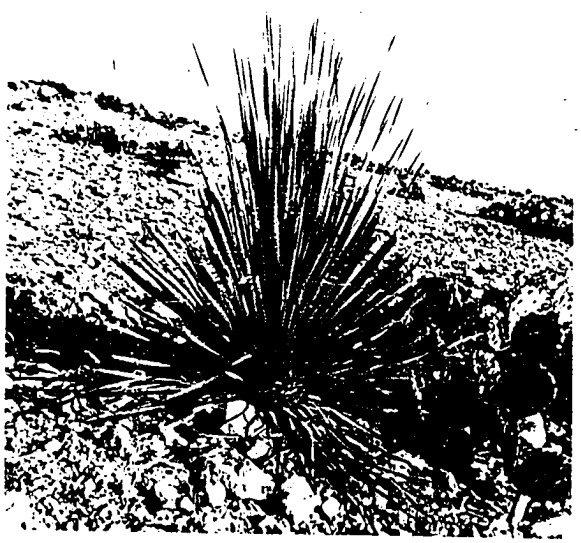
Figure 9. Photograph of a very large adult plant, most of the dead leaves of which have been removed in order to expose the stem. This stem is approximately 24 inches high and about 6 inches wide at its base. This type of stem is usually present in plants similar to the one in figure 2. Site is near Sanderson, Texas. Note the extensive, but shallow, root system.

Figure 10. Habit photograph of a medium-sized plant. This plant is approximately 24 inches tall. Site is near Alpine, Texas. Note the rosette of leaves, which is partly subterranean.



that the condition is actually due to branching (fig. 5). Arber (1925) points out that, compared to dicotyledonous axes, those of monocotyledons are poorly branched. She believes that this is a mechanical effect of the abbreviation of the axis and its enclosure within a succession of leaf sheaths. She states further that these factors result in a lack of space for bud development, and that the formation of branches is thus confined and hindered.

In seedlings and young plants the stems are usually subterranean. In medium-sized adults, however, they often appear only partly subterranean (fig. 10). In much older plants, the axis is almost wholly above the level of the soil. If a large plant is pushed over on its side (fig. 11), the shallow root system is found to be the only connection with the soil. When the dead leaves are removed (fig. 9) the relationship of stem and roots is apparent. The primary root system of Dasylirion (fig. 20) is ephemeral, as in many monocotyledons (Arber, 1925), and is replaced by long-lived adventitious roots after a few weeks (figs. 6, 12). Figure 13 shows the adventitious root system and part of the axis of a young adult plant about the size of that in figure 1. The root surface shows a curious transverse wrinkling (compare with figure 12). According to Arber (1925), wrinkling occurs in the outer cortex in many monocots and is involved in the shortening of the roots. In Dasylirion, the number of roots is increased by the annual development of a fresh crop

Figure 11. Photograph of an adult plant which has been pushed over on its side, showing the mass of dead leaves which surrounds the stem. This plant is about 5 feet tall. Site is near Sanderson, Texas. Note the shallow root system.

Figure 12. Photograph of seedlings showing adventitious roots. Note the young and the older adventitious roots. The distichous arrangement of the leaves is also evident.

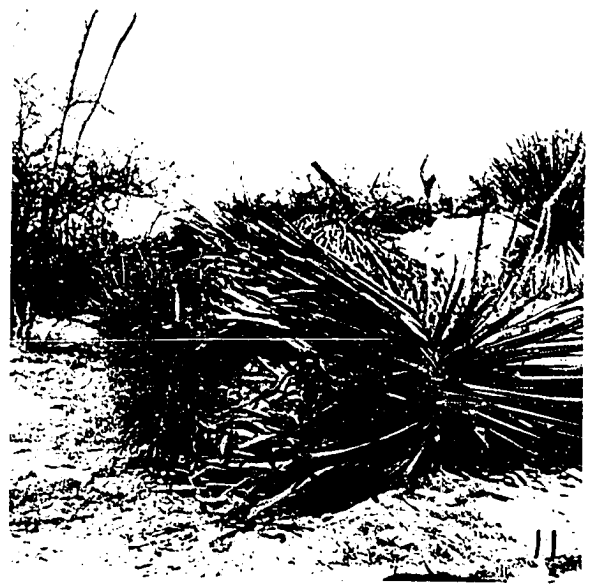



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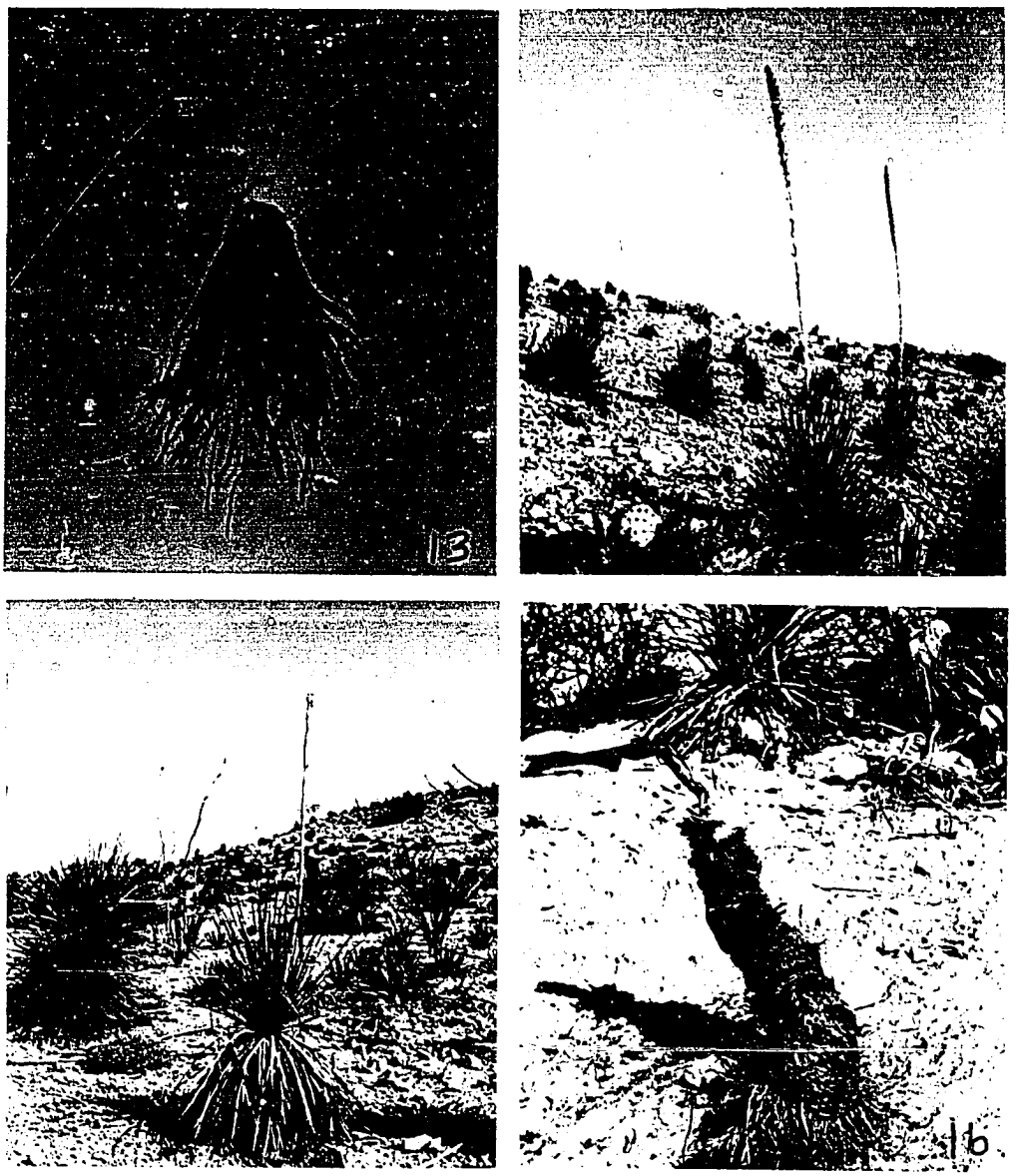
Figure 13. Photograph of a medium-sized plant, the leaves of which have been removed, showing the upper part of the stem and the numerous, fleshy adventitious roots. This specimen measured 140 mm from the tip of the longest roots to the summit of the axis. The axis was 35 mm in diameter at its widest point. As the roots die, the cortex falls away, leaving the wiry, central core of the stele.

Figure 14. Habit photograph of an adult plant bearing a flowering stalk with fruit. The stalk is approximately 7 feet tall. Site is near Alpine, Texas.

Figure 15. Habit photograph of an adult plant with a flowering stalk, the upper part of which has been broken off. Usually, the remainder of the stalk breaks next close to the tip of the stem, as shown in figure 16.

Figure 16. Photograph of the stem of an adult plant showing part of the flowering stalk at its tip. This is the same stem as the one shown in figure 9, with the leaves removed.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outside the older ones, each set being thicker than those of the preceding year.

Coulter (1891-94) states that Dasylirion is dioecious, with flowers in dense racemes, forming a narrow compound panicle. The perianth segments are oblong-obovate obtuse; the stamens are exserted; the style is short; and the triangular, three winged, one celled, one-seeded, coriaceous, indehiscent, fruit has an obtusely triangular seed. The unbranched flower stalk is 5 to 15 feet tall and produces a long, dense panicle of small, white flowers at its upper end. Figure 14 shows a plant with flowering stalk and fruit. In figure 15, the part of the stalk bearing the fruit has been broken off. Eventually, the remainder of the stalk breaks off close to its attachment to the axis (fig. 16). When all leaves are removed from the axis, the places where the flowering stalks were attached appear as holes (fig. 17). Apparently, when the flower stalk dies, the death of tissue extends a certain distance back into the stem. Infloresences are terminal structures (fig. 16), and when they die axillary buds at the base of the stalk begin growth and gradually produce a new head. Growth of the vegetative axis is thus sympodial.

CHAPTER III

METHODS AND MATERIALS

Specimens of Dasylirion leiophyllum were collected in April and December of 1957 from two locations in Texas. One of these was a rocky hillside ten miles east of Alpine on Highway 90, a location especially good for seedlings, young plants, and medium-sized plants. Seedlings at this location were growing in protected spots such as rock crevices and clumps of low, shrubby vegetation (fig. 18). The other site was a rocky, hilly section ten miles east of Sanderson, also on Highway 90. Seeds and much larger plants were collected here.

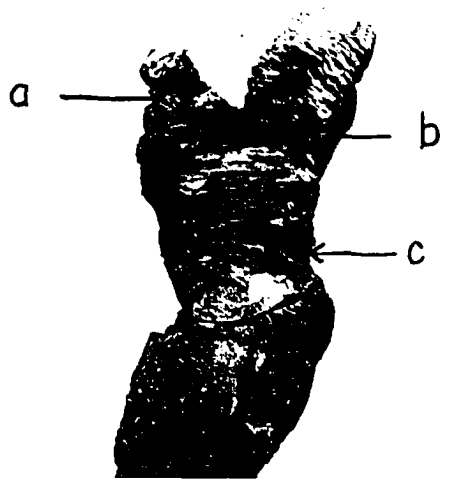
In order to have very young seedlings for study, seeds were allowed to germinate on moist filter paper in a glass germinator. As germination progressed, seedlings with cotyledon-hypocotyl axes from one-eighth to one-half inch in length (fig. 19) were selected, dissected, and preserved to provide material for study of initiation of the first leaf and early activity of the primary thickening meristem. Many seedlings with cotyledon-hypocotyl axes from one-half to three-fourths inches in length (fig. 20) were transferred to soil in clay pots, where they grew into larger plants (fig. 21).

Figure 17. Photograph of the plant shown in figure 5, after the leaves have been removed. The widest part of this stem is about 4 inches. Note the holes at a, b, and c, which mark the places where previous flowering stalks originated.

Figure 18. Habit photograph of a seedling growing in a protected spot. The seedling is approximately 10 inches high. Seedlings were never found growing in the open, unprotected. Site is near Alpine, Texas.

Figure 19. Photograph of seeds which have germinated, showing emergent seedlings. In "a" the hypocotyl and part of the cotyledon have grown out of the seed. The tip of the cotyledon remains in the seed and absorbs food. Note the bend in the cotyledon where it emerges from the seed in "c".

Figure 20. Photograph of seedlings showing emergence of the first leaf. In "a" the leaf has not emerged. The root shown is the primary root, and the long, slender structure coming from the seed is the elongated cotyledon.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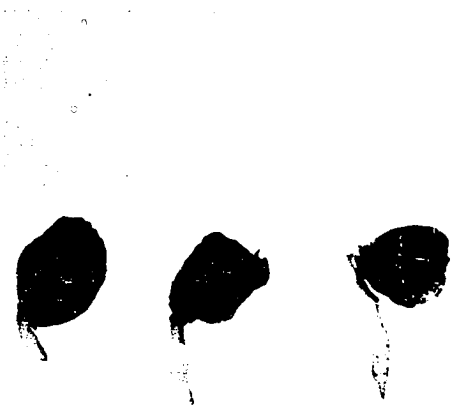
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a
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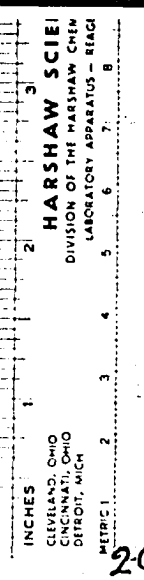
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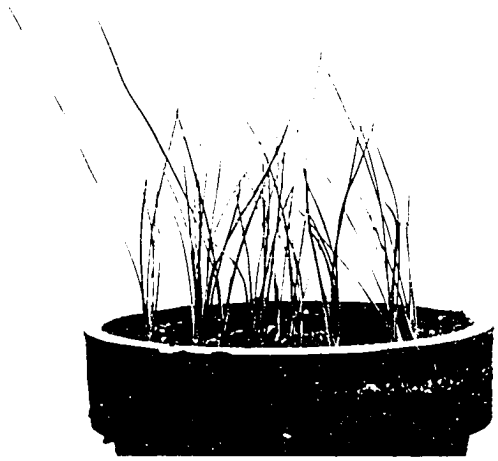
Specimens were selected weekly for 16 weeks to provide material for study of the development of the apical meristems, primary thickening meristem, and leaf initiation. After the sixteenth week seedlings were selected at 18, 24, 28, 30, 38 and 42 weeks. In the case of emergent seedlings up to one-fourth inch in length (fig. 19), dissection consisted merely in cutting the cotyledon at the point where it emerged from the seed coat. Cylinders of tissue were removed from somewhat larger seedlings (figs. 20b, c) by cutting across the cotyledon just above and below the cotyledonary plate. This latter method was used until the seedlings were about 16 weeks old. After 16 weeks, the seedlings were sufficiently large (fig. 6e) that small blocks of tissue containing the young stem could be easily dissected from the basal part of the plant.

For the study of older seedlings (fig. 7), medium-sized plants, and adult plants, the leaves and roots were first removed from the axis, after which the stem tip (fig. 22) was excised. Small blocks of tissue, about 5 mm square, were then dissected from the center of the stem to provide material for the study of the apical meristem. Transverse sections of stems were made from cylinders of tissue obtained from stems 7-8 mm in diameter.

Material for embryonic study was obtained by dissecting the embryos from seeds which had been soaked in water for 12 hours.

Figure 21. Photograph of seedlings as they were grown in the greenhouse. The longest leaves are approximately 8 inches.

Figure 22. Photograph of the upper surface of the rounded tip of the stem of an adult plant. This came from the stem shown in figure 17. The outlines of the leaf bases can be seen encircling the stem. In the center is a depression in which the apical meristem is situated.



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FAA and Craf V (Sass, 1940) were used for killing and fixing. Most of the material, however, was fixed in Craf V. After 48 hours in the fixing solution, the material was dehydrated using a variation of the tertiary butyl alcohol method (Johansen, 1940).

Transverse and longitudinal serial sections of apices were cut at 8μ with a power-driven rotary microtome. Cross sections of woody stems, however, were cut at 14μ. Most of the sections were stained with a variation of Conant's quadruple stain (Johansen, 1940) and a mordant; with some, however, a variation of Johansen's triple stain (Johansen, 1940) was used.

Plants were photographed in the field with a Rollicord reflex camera and 120 verichrome black-and-white film. Many close-up views of seedlings and plant parts were made with the same camera with parallax correction lenses attached. Some close-ups, however, were made with an EXAKTA camera with close-up attachments and 35 mm film.

Photomicrographs were taken on Eastman Panatomic X film. In most cases an orange filter was found to improve resolution and contrast.

CHAPTER IV

RESULTS

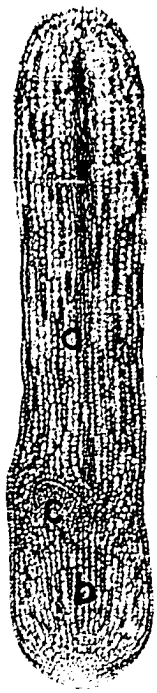
Structure and Development of the Apical Meristem

The Embryonic Apex

In median longisection, the meristem of the embryo appears as a small cluster of cells at one side of the much larger primordium of the first leaf (figs. 23, 24). The two structures constitute the epicotyl and are situated in the cotyledonary slit, surrounded by the sheathing base of the cotyledon. In a transection of the embryo about midway between the summit and base of the epicotyl (fig. 26) the apical meristem appears as the inner, darker-stained portion. Figure 25 represents a longisectional view at right angles to that shown in figure 24; here the meristem appears as a low mound between procambial strands. Zonation in the embryonic apex is inconspicuous. There is an outer layer (figs. 24, 25) and a central area; the former probably represents cells from which the tunica will develop, while the latter appears to be the place of origin of the corpus. The cells of the apex are in general less vacuolate than the surrounding cells and therefore stain somewhat more intensely.

Figure 23. Near-median longisection of the embryo. (a) cotyledon (b) hypocotyl (c) epicotyl. The embryo is a cylinder of almost uniform diameter except in the region of the cotyledonary slit, where it bulges slightly. Note that most of the embryo consists of cotyledon; the one shown here is 2337 μ long and 252 μ in diameter. x35.

Figure 24. Median longisection of the embryo in the region of the cotyledonary slit. (a) epidermis (b) cotyledon (c) procambium (d) cotyledonary slit (e) sheath of the cotyledon (f) leaf primordium (g) embryonic meristem (h) stem (i) hypocotyl. x224.



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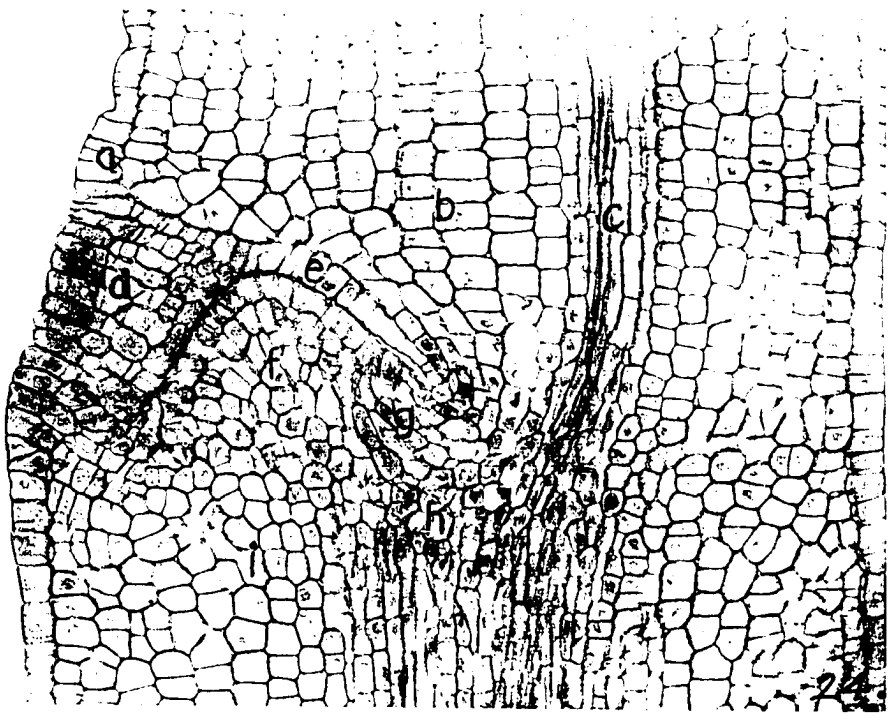
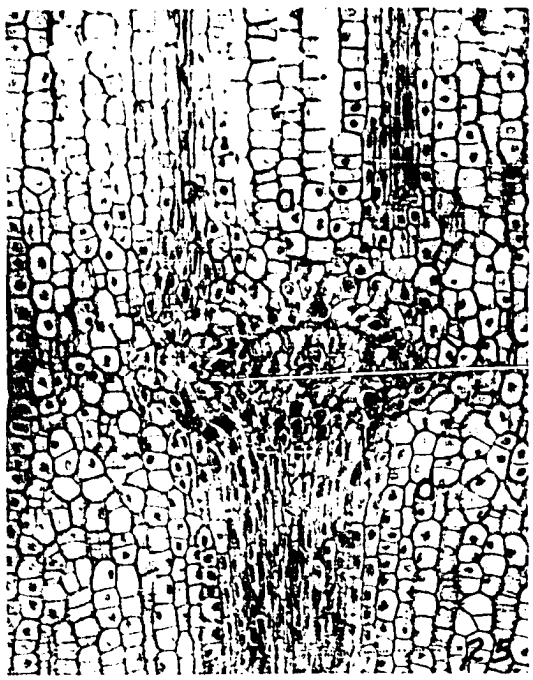


Figure 25. Longisectional view of the embryonic meristem at right angles to the plane of the cotyledonary slit. (a) cotyledon (b) procambium (c) meristem (d) hypocotyl. Note how the outline of the meristem in this view differs from that in figure 24. x176.

Figure 26. Transection of the embryo about midway between the summit and base of the epicotyl. (a) epidermal layer (b) cotyledonary slit (c) cotyledon (d) sheath of cotyledon (e) leaf primordium (f) embryonic meristem (g) procambium. x176.

Figure 27. Near-median longisection of an emergent root tip 3 mm long. (a) cotyledon (b) young leaf (c) hypocotyl. The cotyledon was cut at the point where it emerged from the seed. x35.

Figure 28. Near-median longisection of the epicotyl of an emergent seedling 3 mm long. The apical meristem is on the lower left, at the side of the primordium of the first leaf, a. The cells within the developing shoot apex are dividing in various planes. Note the elongation of the cells below the apex. Procambium is visible in the leaf primordium and young axis. x145.



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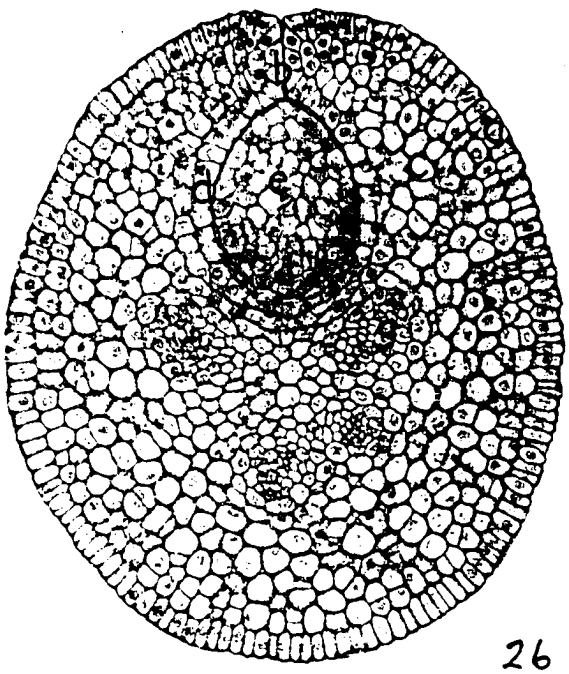

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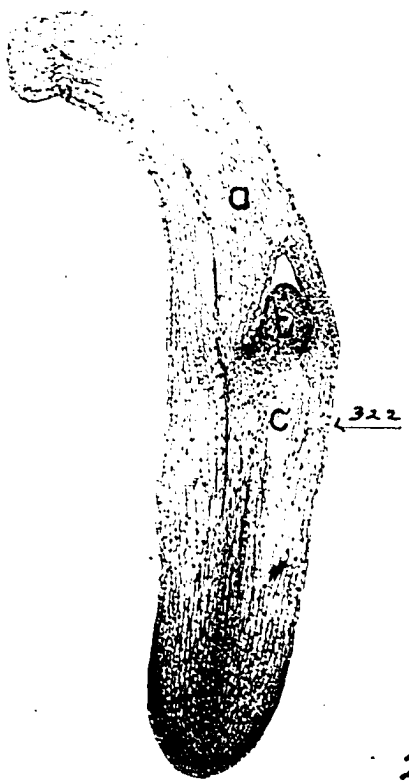

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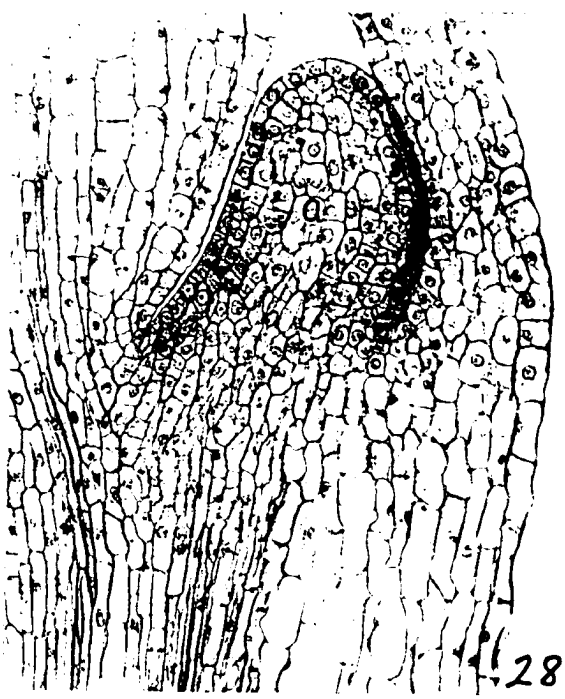

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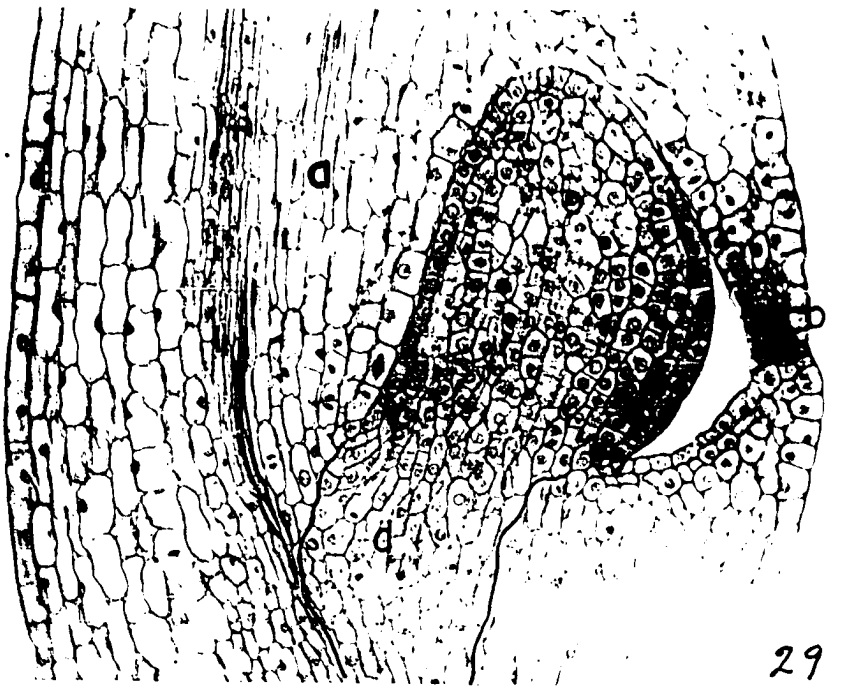
When the emergent seedling has reached a length of 3 mm (fig. 27), the leaf primordium is approximately 336 μ high and the developing shoot apex, near its base, is much more conspicuous than it is in the embryo. In figure 28, cell divisions have occurred in the region of the meristem referred to as the central area in the embryonic apex, and below this area cells are undergoing elongation. Apparently, the divisions in the central area (fig. 28) result in the establishment of cells which, due to their position in the young apex, resemble corpus initials (fig. 29). The files of cells below the presumtive corpus initials probably represent the beginning of the rib meristem. In a transectional view 16 μ below the base of the young leaf (fig. 30) the outlines of cells in this area can be clearly distinguished from the surrounding cells.

In figure 19c the seedling is about 12 mm long. At this stage, several significant changes in the development of the apical meristem are evident in median longisections. In figure 31 the apex is almost flat. Another significant change in the meristem at this stage of development is the appearance of more conspicuous zonation. There appears to be a 1-layered tunica below which are several corpus initials. The beginning of a flank meristem, consisting of 2 layers of cells, is evident to the left of the tunica and corpus. A fairly distinct rib meristem occurs below the corpus. In figure 32, which represents a somewhat later stage of

Figure 29. Median longisection of a 3-mm long emergent seedling in the region of the cotyledonary slit. (a) cotyledon (b) cotyledonary slit (c) developing apical meristem (d) young axis. Note the cells which are, or will become the rib meristem. x152.

Figure 30. Transection of an emergent seedling 3 mm long, 16 μ below the base of a leaf. (a) derivatives of the corpus (b) procambium. x228.

Figure 31. Median longisection of the developing apical meristem of an emergent seedling 12 mm long. The arrow points to the 1-layered tunica, below which are three cells which probably represent the corpus initials. Cells at the flanks of the presumed corpus initials may represent the beginning of the flank meristem. x226.



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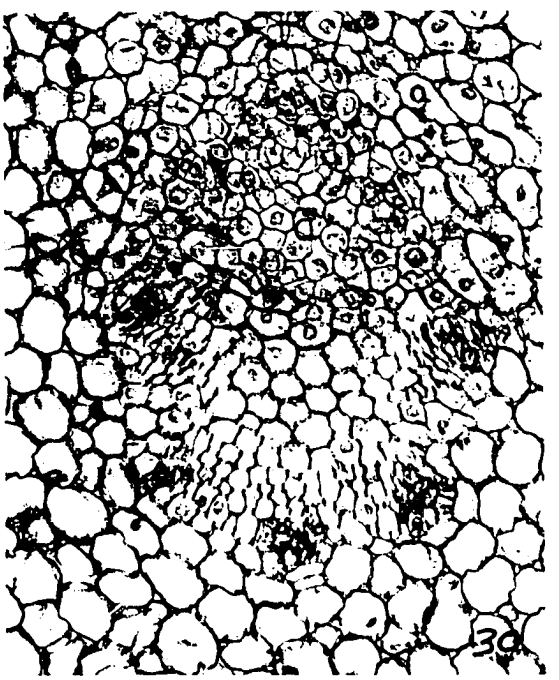

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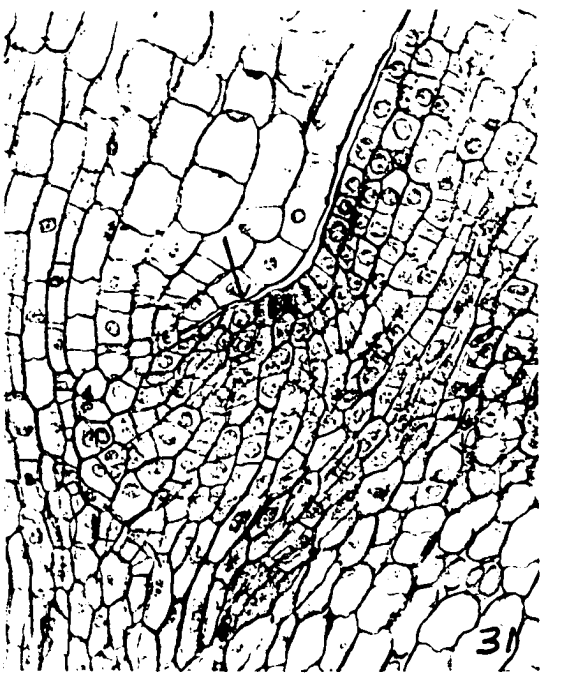

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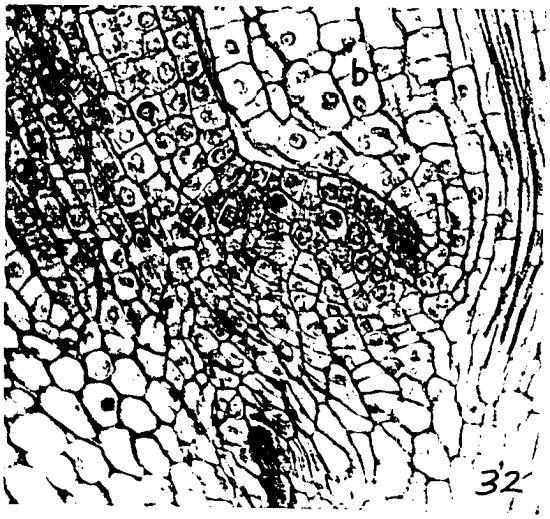
Figure 32. Median longisection in side view of the developing apical meristem of an emergent seedling 12 mm long. (a) primordium of the first leaf (b) cotyledon. Presumably, this meristem represents a later stage than the one in figure 31. Note the rib meristem and its derivatives. x226.

Figure 33. Median longisection in side view of an early dome-shaped apex with conspicuous zonation. Note the cambium-like zone below the rib meristem and the numerous divisions in the flank meristem. x226.

Figure 34. Median longisection in side view of part of an emergent seedling 12 mm long, showing cotyledon, first leaf, apical meristem and axis. The cotyledonary sheath has elongated, keeping pace with the developing leaf. x28.

Figure 35. Near-median longisection in side view of the early apical meristem of an emergent seedling 12 mm long. Note the numerous divisions in the flank meristem and the area occupied by zone 5. x228.

Figure 36. Median longisection of the apical meristem in face view of an emergent seedling 12 mm long. Zone 5 is just below the third layer of the flank meristem. x228.



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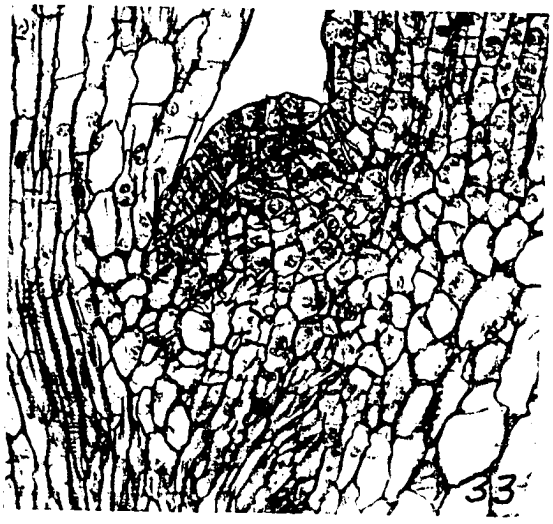

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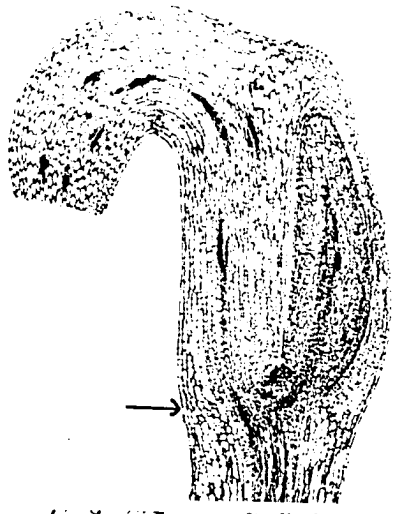

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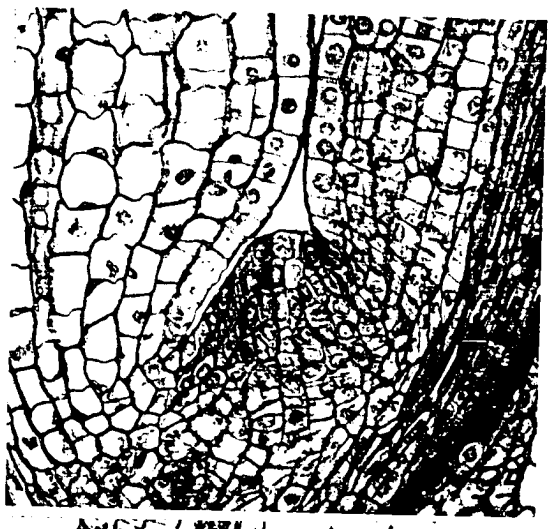

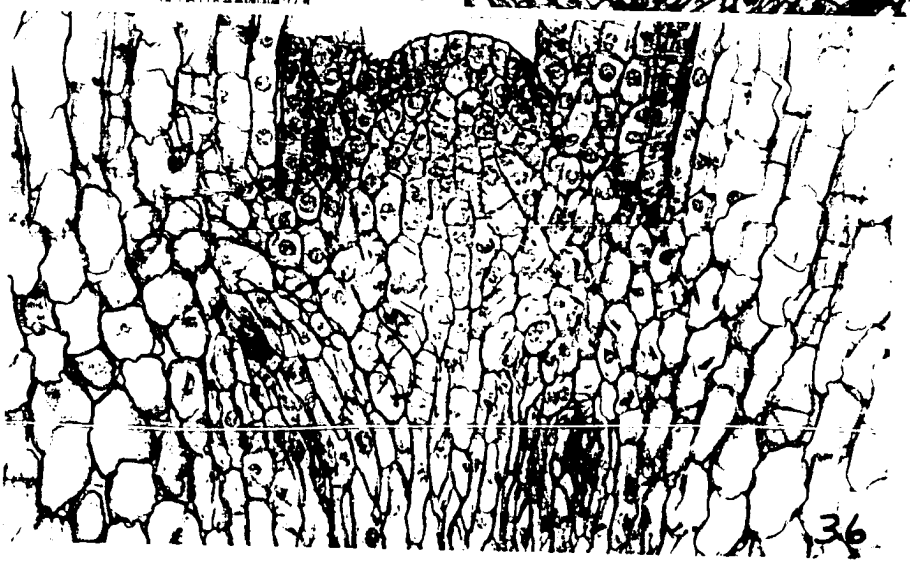

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development, the earliest apical dome is visible. This results from increased anticlinal divisions in the surface layer along with an increase in the number of periclinal divisions in the rib meristem. Figure 33 probably represents a stage in the development of the apex shortly before initiation of the second leaf. By the time the first leaf is about 1.25 mm long (fig. 34), the part of the seedling consisting of the hypocotyl-axis and the enlarged part of the cotyledon has reached a length of approximately 12 mm (fig. 20a). At this stage the shoot apex is a low mound at the base of the young leaf (fig. 35).

The Juvenile Apex

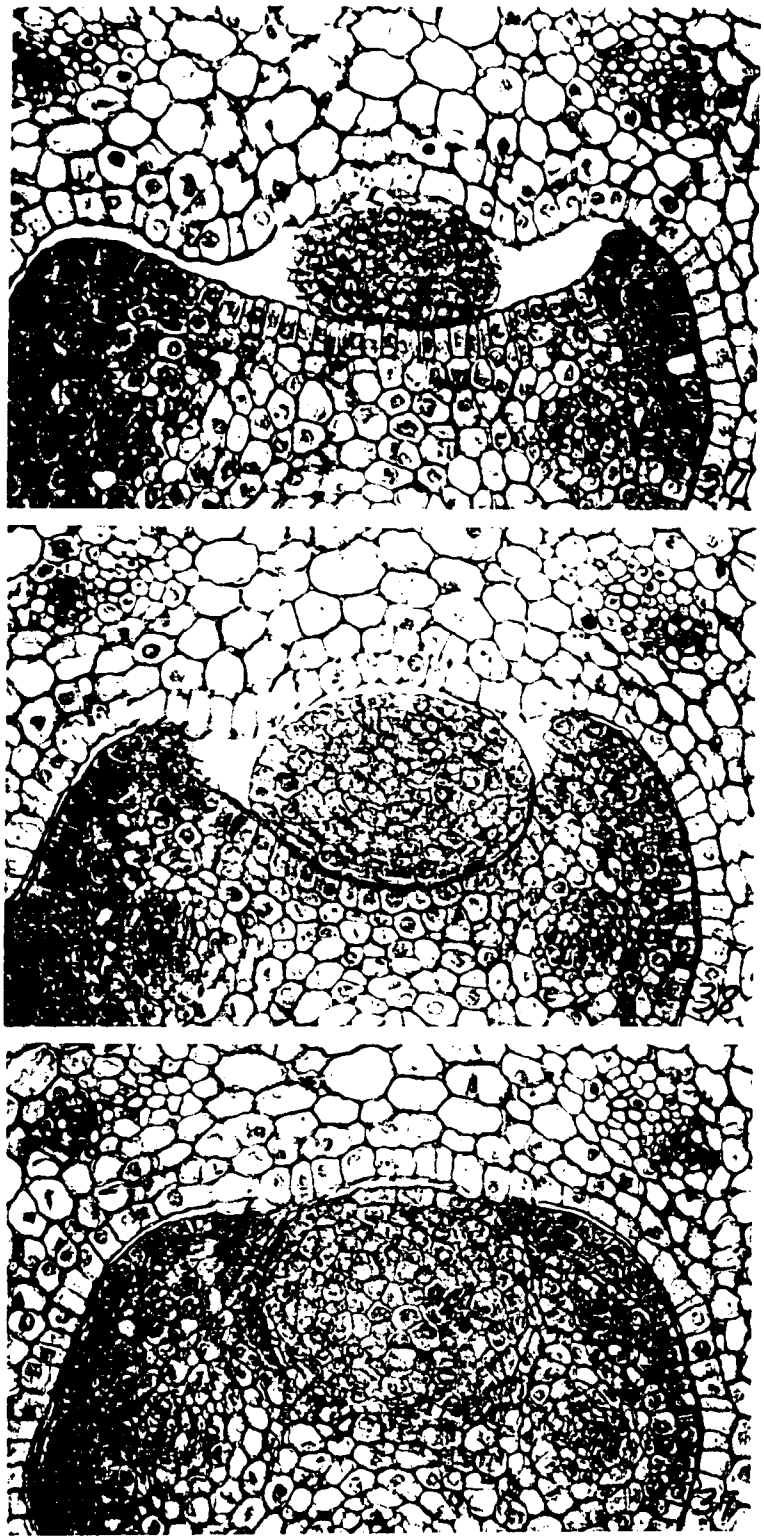
In order to obtain an accurate picture of the general shape of the early apical meristem at maximum area, it is necessary to consider both side and face views; the former refers to sections made in the plane of the cotyledonary slit, while the latter includes those made at right angles to it. In a side view (fig. 35) the apex is often a narrow dome, somewhat flattened at its summit; a face view, however, (fig. 36) reveals that the top is broadly rounded. Transectional views at 16 μ below the summit (fig. 37), and also midway between the summit and the base (fig. 38), show that the outline at these levels is elliptical; the base, however, (fig. 39), is almost circular. Measurements on the specimens examined reveal that the apical dome has an average height

Figures 37 through 39 represent transections at different levels of the apical meristem of an emergent seedling 12 mm long.

Figure 37. A transection 16 μ below the summit of the apical meristem. In the center the outlines of the corpus cells are visible, surrounded by cells of the tunica. x228.

Figure 38. A transection midway between the summit and the base of the apical meristem. The cells in the center probably represent cells of the lower corpus, and are surrounded by cells of the flank meristem. x228.

Figure 39. A transection just above the base of the apical meristem. Cells of the rib meristem are visible in the center, directly above which are cells which represent the lower part of the flank meristem. x228.



and width of 33 and 99 μ respectively. These measurements included only the region above the line indicated in figure 33.

Since initiation of the second leaf usually occurs shortly after the meristem reaches the stage shown in figure 35, this early form does not persist. However, during this period zonation is conspicuous. Five zones are delimited in figures 49a and b, which are diagramatic representations made from several series of slides. Zone 1 is a 1-layered tunica in which no periclinal divisions have been observed. The cells of this layer are relatively large, highly vacuolate, and more or less uniform in size. Laterally, zone 1 contributes cells to the first layer of the flank meristem. Zone 2, the corpus initial zone, occupies the center of the apical meristem directly below the tunica, and appears to consist of 2 tiers of cells which closely resemble the cells of the tunica in size, shape, and vacuolation but which differ in planes of division, which are diverse. However, the features of corpus cells fluctuate during the plastochron (Gifford, 1950), as is evident in figures 42, 43, and 44, which represent stages in the development of the second leaf. Laterally, zone 2 contributes cells to the second and third layers of the flank meristem, while at its base it gives rise to cells which become the rib meristem (zone 4). As stated above, both tunica and corpus contribute cells to the flank meristem (zone 3); this is a zone consisting of 3 layers of

Figure 40. A near-median longisection of an emergent seedling 12 mm long. Compare with figure 34 and note how the two meristems differ in size. The first leaf is still surrounded by the cotyledonary sheath. x28.

Figure 41. A detailed view of the meristem shown in figure 40. Note the numerous divisions in the flank meristem associated with the initiation of the second leaf. x228.

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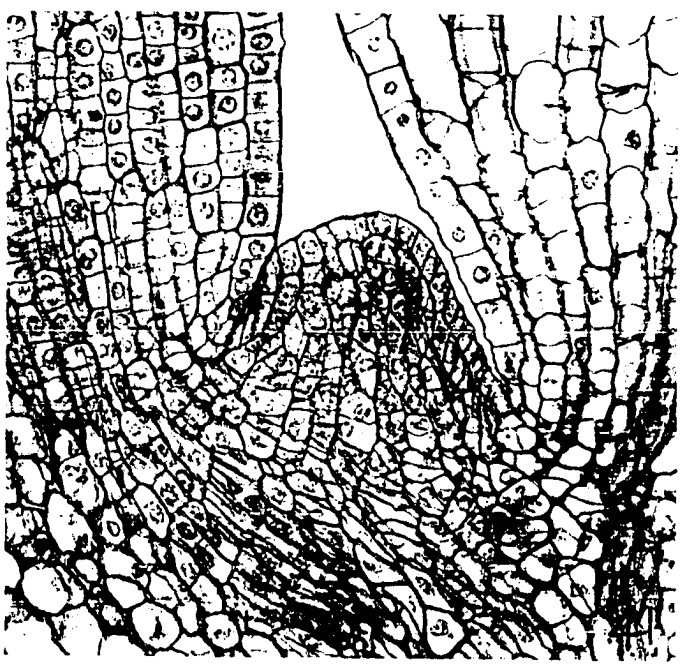


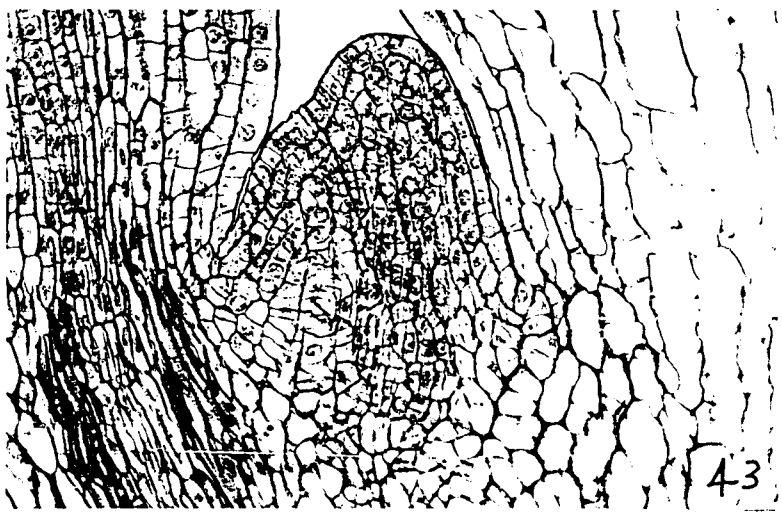
Figures 42 through 44 represent near-median longisections of apical meristems of seedlings 2 weeks old in side view. In each illustration, the primordium of the second leaf overtops the apical meristem.

Figure 42. This section shows the apical meristem as it appears at an early stage in the development of the second leaf. Compare the outline of this meristem with the one in figure 24. Note the continuation of the three layers of the flank meristem into the leaf primordium. x226.

Figure 43. The leaf primordium in this section is 12 μ higher than the one in figure 42. Note the periclinal divisions in the corpus and protoderm of the leaf. x226.

Figure 44. In this section the primordium shows still further development. Note the periclinal divisions in the corpus and the numerous divisions in the rib meristem. x226.

42



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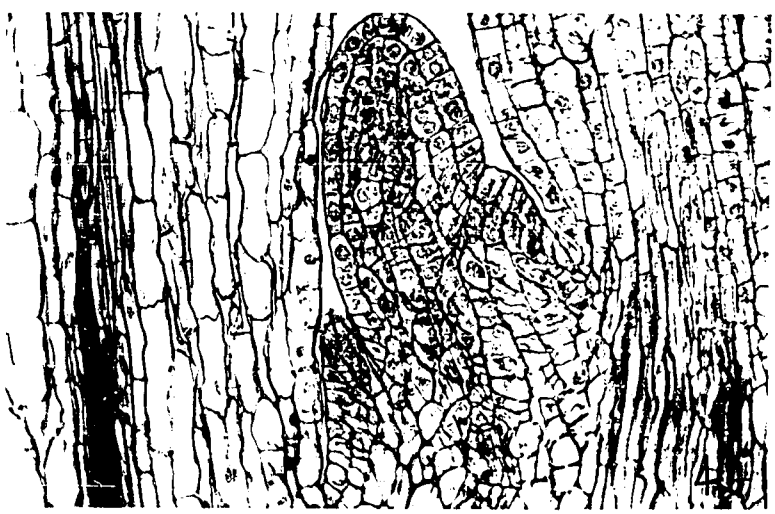

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smaller, less vacuolate, and denser staining cells. Cell divisions in this zone are much more frequent than in the corpus and tunica, and the planes of division are generally oblique with respect to the axis. Zone 3 produces the epidermal system of the shoot and contributes cells to the internal tissue of the leaf; it is also involved in leaf initiation. Zone 4 is a rib meristem. It originates at the base of zone 2 and consists of highly vacuolate, lightly staining cells arranged in columns. The planes of division at the origin of the rib meristem are various, but a short distance below they are mostly transverse, although divisions at oblique angles to the axis are rather common. Figure 39 is a transection of the apical meristem just below the corpus initials, showing the outlines of cells making up the upper rib meristem. Derivatives of the rib meristem contribute to the central core of the axis. The immediate derivatives of the corpus initials contribute cells laterally, the derivatives of which form zone 5 (fig. 35). This area is situated below the flank meristem and lateral to the rib meristem; its cells are rather large, highly vacuolate, and light staining, and divisions may occur in diverse planes. Vascular bundles form in this zone near the apical meristem and the primary thickening meristem originates in its derivatives which spread out below the leaves.

In the early stages of initiation of the second leaf (figs. 40, 41) the meristem shows an increase in size, but as

leaf development continues it becomes almost flat (fig. 42), and resembles that in the embryo (fig. 24). Zonation, however, is more conspicuous than it is prior to initiation of the second leaf. The corpus zone is stratified (fig. 42), but as development of the leaf procedes (figs. 43, 44) the stratification is disturbed by divisions in various planes. As the young leaf continues to elongate the shoot apex gradually regains its original shape. Considerable disorganization appears to accompany this process, especially in the corpus.

At the age of 4 weeks, a side view of the shoot apex at maximum volume appears in median longisection as a steep cone, slightly flattened at its summit (fig. 45). The tunica now consists of 2 layers of cells, more or less uniform in size, shape, and staining. Zone 5 is visible as a narrow area below the flank meristem, and its cells are dividing in diverse planes. The apex in figure 45 is 79μ high and 112 μ wide. The apex of a 6-weeks' old seedling in side view is almost identical with that of a 4-weeks' apex, and in face view (fig. 47) it is dome-like in outline with almost vertical sides; T-1 and T-2 are conspicuous features. Figure 49d is a diagramatic representation of a face view of the apex at 6 weeks.

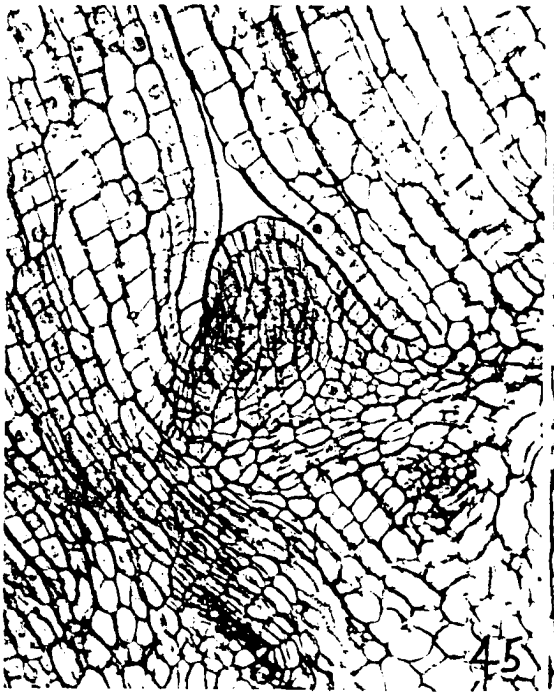
Figure 46 represents a median longisection of the shoot apex of an 8-weeks' seedling in side view, and probably is near maximum volume; it appears to differ from the 4-weeks' apex (fig. 45) only in the outline of the summit,

Figure 45. A median longisection of the apical meristem of a 4-weeks' old seedling, as observed in side view. Note the 2 tunica layers and the corpus, the upper layer of which is stratified. x228.

Figure 46. A median longisection in side view of the apical meristem of a seedling 8 weeks old. Note the 2 tunica layers and the corpus, the upper layer of which is stratified. Note also the periclinal division in the second layer of the flank meristem. Zone 5 is visible inside and below the flank meristem. x228.

Figure 47. A median longisection in face view of the apical meristem of a seedling 6 weeks old. Note the 2 tunica layers and the corpus zone beneath. x228.

Figure 48. A median longisection of the apical meristem of a seedling 15 weeks old as observed in face view. Compare this apex with the one in figure 47. Development of the meristem appears to have been mostly in a plane at right angles to the cotyledonary slit. Note the increase in the number of upper corpus cells and their stratification, which causes them to appear to constitute a third tunica layer. Note that zone 5 is better developed but still originates from the loosely arranged cells of the corpus. x224.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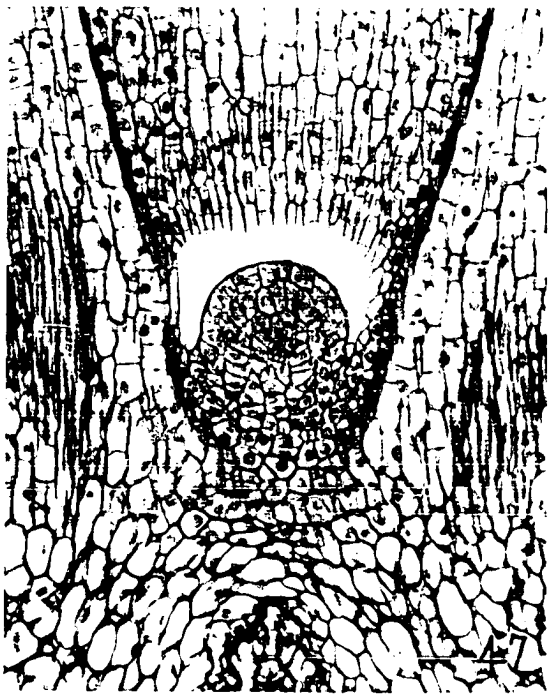
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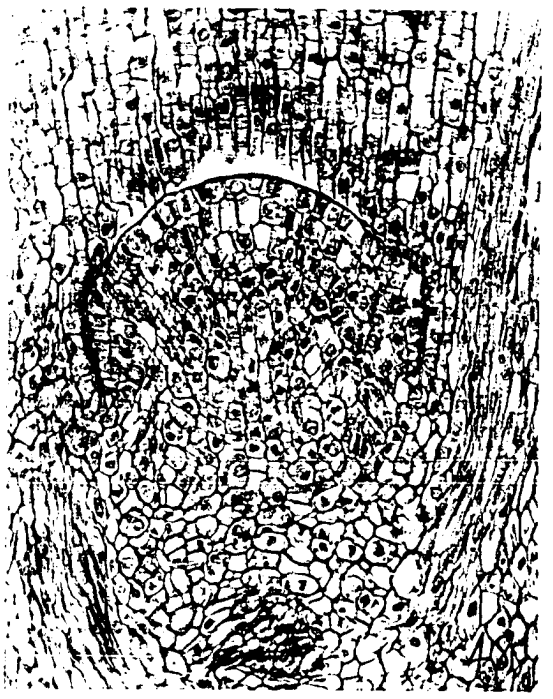
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Figures 49a-h represent diagramatic interpretations of the apical meristems of plants of various sizes. Zones are numbered.

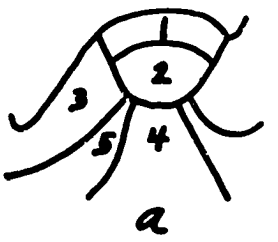
Figures 49a, b. Emergent seedlings 12 mm long in side and face views, respectively. x228.

Figures 49c, d. Seedlings 6 weeks old in side and face views, respectively. x228.

Figures 49e, f. Seedlings 15 weeks old in side and face views, respectively. x228.

Figure 49g. Plant with a stem 7 mm wide and 10 mm long. Side view. x224.

Figure 49h. Plant with stem 10 mm wide and 10 mm long. Face view. x226.



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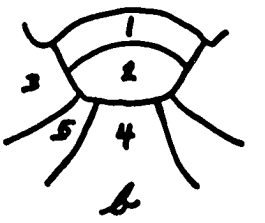

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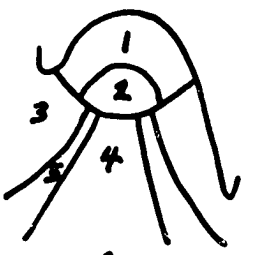

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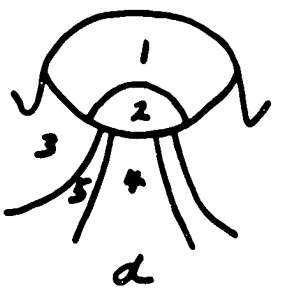

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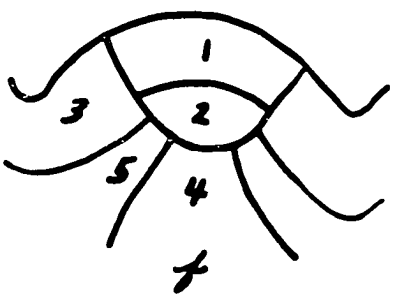

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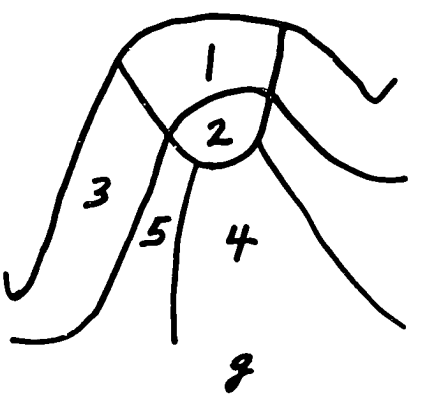

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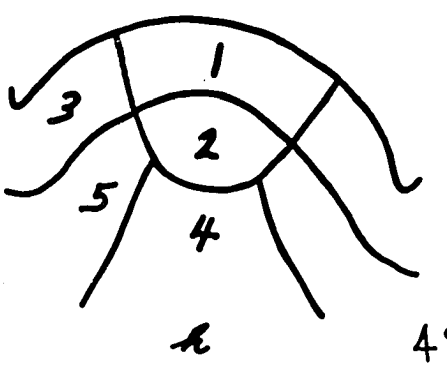

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which is more pointed. Periclinal divisions have not been observed in T-1, but they usually occur in T-2 during leaf initiation and development. Figure 49c is a diagramatic representation of the apex in side view as it appears in seedlings 4 to 8 weeks old. The first 2 layers of the flank meristem now originate from the tunica, while the third layer still comes from the corpus. The second tunica gradually is formed as anticlinal divisions in the outer corpus cease.

By the time the seedling is 15 weeks old (figs. 6a, b, c), the outline of the shoot apex in side view and at maximum volume is that of a low cone (fig. 49e); in face view, however, (figs. 48, 49f), it is a broad dome. The apices in figures 46, 47, 48, and 49e are all at about the same magnification; if those in figures 48 and 49e are compared with those in figures 46 and 47, it becomes evident that growth in the developing meristem has occurred predominantly in a plane at right angles to the cotyledonary slit. The apex in figure 47 is 85 μ wide and 59 μ high, while the one in figure 48 is 168 μ wide and 56 μ high. Figures 49e and f are diagramatic representations of the 15-weeks' old meristem in side and face view at maximum area.

Figures 51 through 53 represent transections at 3 levels of the apical meristem of a 15-weeks' old seedling. Figure 51, a transection through the tip of the apical dome, shows the outlines of the cells of T-1. At 98 μ below the summit (fig. 52), cells of the lower corpus can be seen in

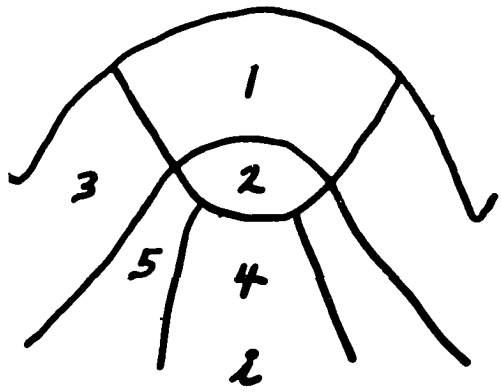
Figures 50i-l represent diagramatic interpretations of the apical meristems of plants of various sizes. Zones are numbered.

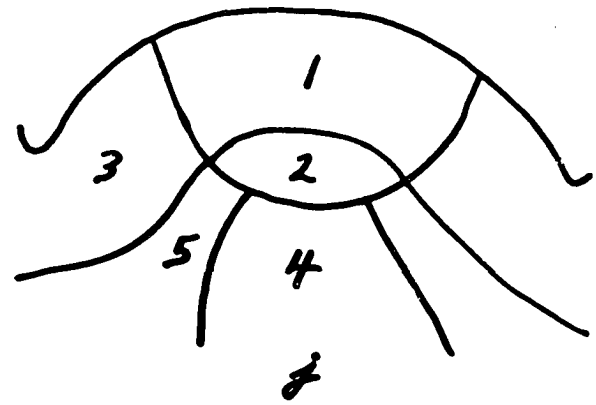
Figure 50i. Plant with a stem 20 mm wide and 20 mm long. Side view. x226.

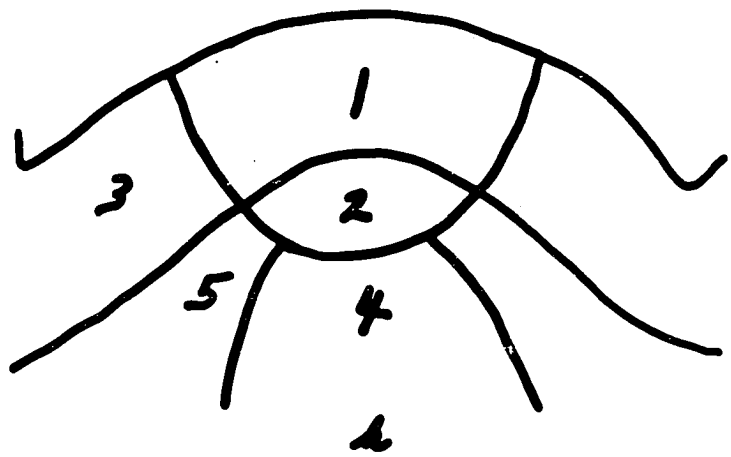
Figure 50j. Plant with a stem 25 mm wide and 25 mm long. Face view. x226.

Figure 50k. Medium-sized plant. x176.

Figure 50 1. Adult plant. x176.







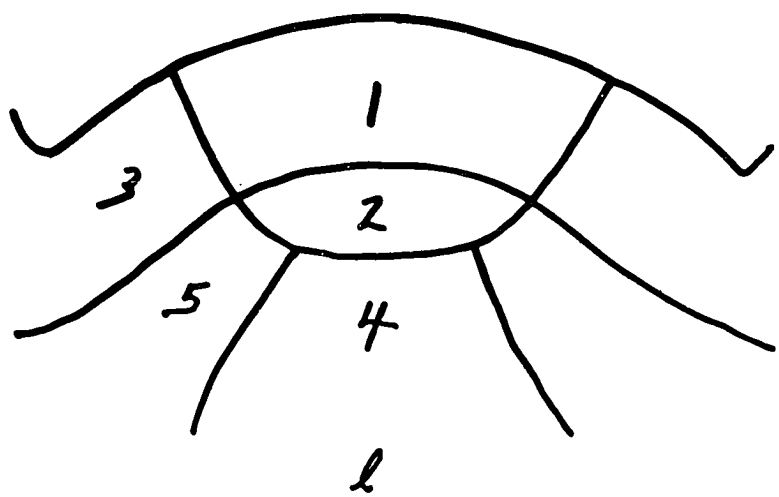


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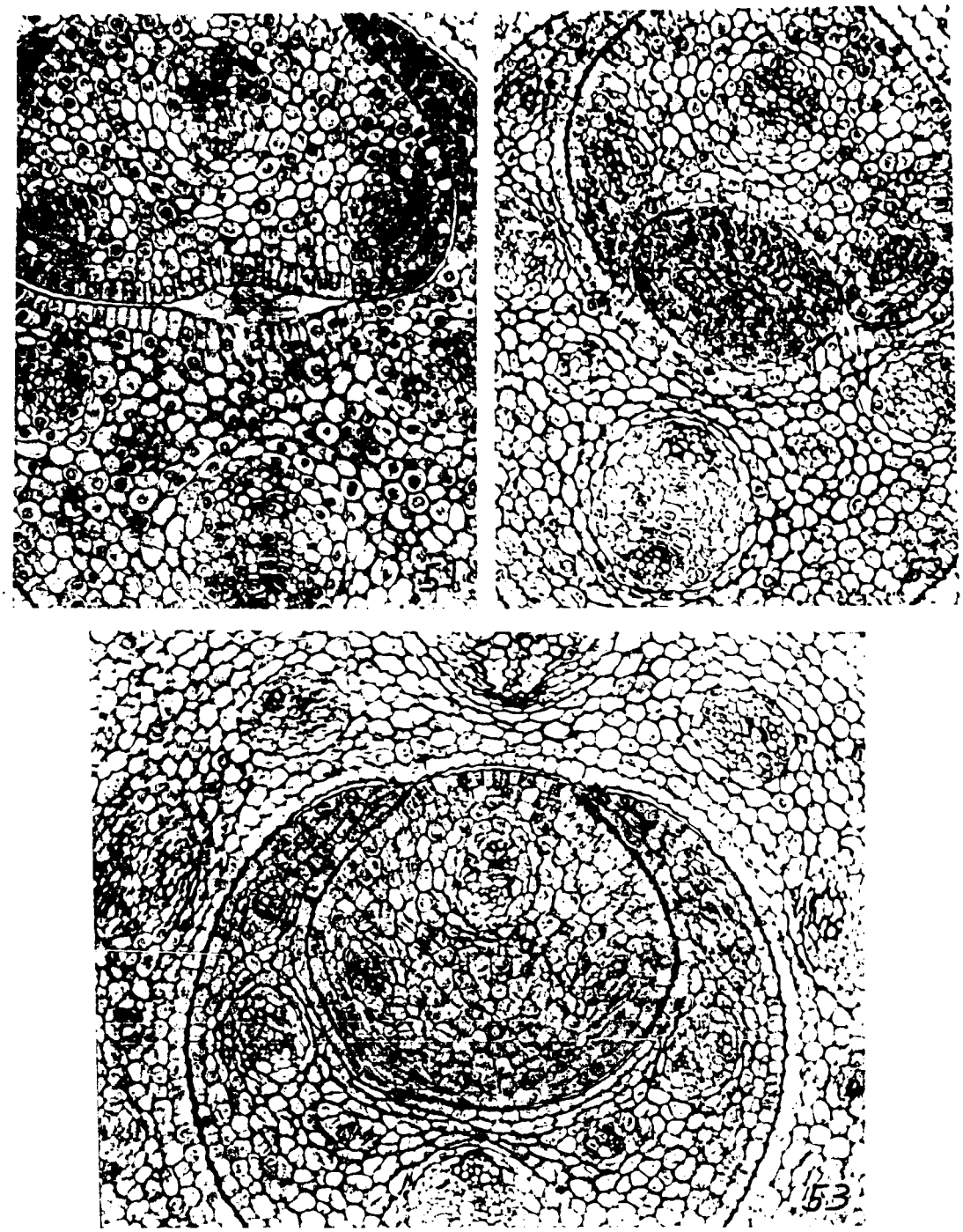
Figure 51. A transection through the tip of the apical meristem of a seedling 11 weeks old. The summit of the apex is still elliptical in outline. Note the outlines of the cells of T-1. x228.

Figure 52. A transection 98 μ below the summit of the apical meristem of a seedling 15 weeks old. This section passes through the meristem just above the base of the long side. Cells of the corpus are evident in the center, surrounded by cells of the flank meristem. The corpus zone as observed in transection is long and narrow. x228.

Figure 53. A transection at the base of the apical meristem of a seedling 15 weeks old. At its base the outline of the meristem is circular. Note how the base of the young leaf encircles the base of the meristem. x228.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the center of the apex, surrounded by derivatives of the flank meristem. At the base of the apical dome (fig. 53) the outline of the meristem is circular.

By the time the seedling is 42 weeks old (figs. 6e, f, g), a side view of the apical meristem appears at near-maximum area as a steep cone (fig. 54); in face view, however, (figs. 55), it is broad and dome-like. The apex in side view is similar to that of the 15-weeks' apex except for size; the former has a height and width of 98 and 140 μ respectively, the latter 70 and 112 μ. Measurements made on the apices in face views (figs. 48 and 55), reveal that they are similar in height but differ in width; the 15-weeks' old apex (fig. 48) has a height and width of 56 and 168 μ, respectively, the 42-weeks' old apex (fig. 55), 56 and 196 μ. The stratification in the upper corpus (fig. 55), as is true in the case of the 15-weeks' old apex, foreshadows the formation of a third tunica layer.

As previously indicated, material for the study of early development of the apical meristem was obtained from seedlings which were grown in the greenhouse. By the time the seedlings were 42 weeks old, they had an average stem diameter of 2.5 mm. The study of cultivated seedlings was discontinued at this stage. The remainder of the investigation is based upon material collected in the field. The smallest plant collected in the field was 6 mm in diameter and 4 mm long. The next largest plant (fig. 7a), was 6 mm

Figure 54. Median longisection of the apical meristem of a seedling 42 weeks old, as observed in side view. The meristem is near maximum area. This apex is undergoing leaf initiation, as evidenced by the periclinal divisions in the flank meristem to the left. The cells of the outer corpus are stratified while those in the lower part are loosely arranged. Compare these loosely arranged cells with the corresponding cells as observed in face view (fig. 55). x228.

Figure 55. Median longisection of the apical meristem of a seedling 42 weeks old, as observed in face view. Note how clearly the third layer of the flank meristem originates from stratified upper corpus cells. Zone 5 is now 2 cell layers in width. Note the tendency toward stratification of the outer corpus layers; this is less obvious in figure 54, because of the narrowness with which the corpus appears in side view. x226.

Figure 56. Median longisection of the apical meristem of a plant with a stem 7 mm wide and 10 mm long, as observed in side view. Note the 3 tunica layers and the lack of stratification in the corpus. x224.

Figure 57. Median longisection of the apical meristem of a plant with a stem 10 mm wide and 10 mm long, as observed in side view. Leaf initiation is occurring on the left. x224.


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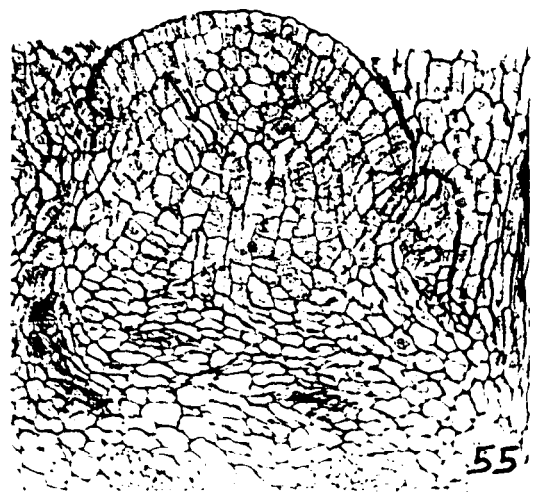
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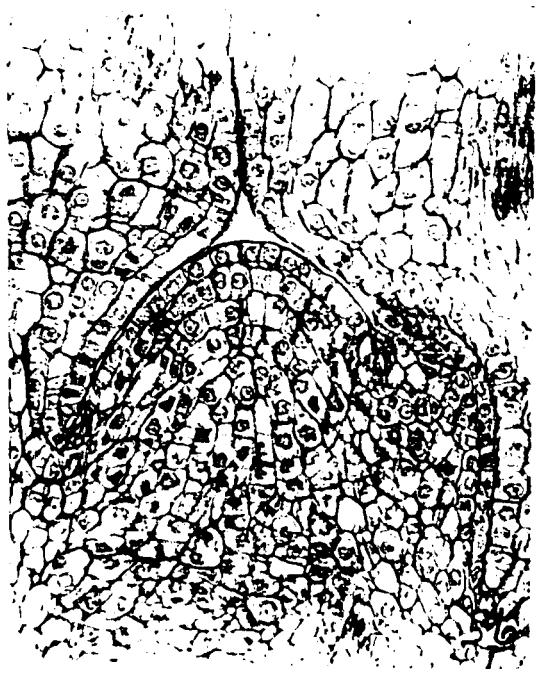
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57

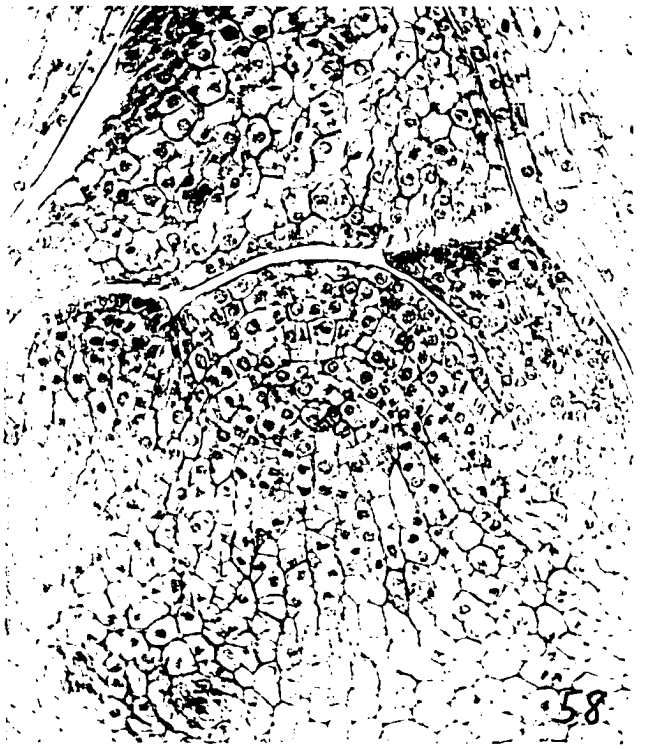
in diameter and 6 mm long. In appearance it was very similar to a 42-weeks' old seedling, but had a much larger stem. Spines were present only at the bases of the leaves. The shoot apex of this plant is not shown, but it is similar to the one in figure 56, which represents the apex of a plant with a stem 7 mm long and 7 mm wide. It is admittedly difficult to ascertain just when the seedling stage terminates. In figure 7a, the plant has no spines except for a few near the base of the leaves; the plant in 7b, however, has spines typical of an adult plant, even though its stem was of the same diameter as the one in 7a and only 2 mm longer. In this study the discussion of the development of the adult apical meristem will begin with the examination of apices of plants with stem diameters of 6-7 mm, regardless of length.

The Adult Apex

Between plants with stems 6 mm in diameter (fig. 7a, b) and those with stems 12 mm in diameter (fig. 7e) the shoot apex shows several significant developmental changes. When the apex in figure 54 is compared with the one in figure 56, it is evident that an increase in size has occurred. When face views are compared (figs. 55, 58) however, the increase is not so conspicuous. The apex in figure 54 is 140 μ wide and 98 μ high, while the one in figure 56 is 168 μ wide and 91 μ high. The meristem in figure 56 shows an increase of 28 μ

Figure 58. Median longisection of the apical meristem of a plant with a stem 10 mm wide and 10 mm long, as observed in face view. The apical dome is more rounded and broader than in a side view. Note how uniform the tunica appears to be in cell size and stratification. x226.

Figure 59. Median longisection of the apical meristem of a plant with a stem 10 mm wide and 10 mm long, as observed in face view. The meristem shown here is at, or near, minimum area, and reveals the expansion that may occur in zone 5 in a plant this size at the time of leaf initiation. Note the anomalous periclinal division that has occurred in T-1, and those on the right under the primordium, which have occurred in the third layer of the flank meristem and in the layer below. x226.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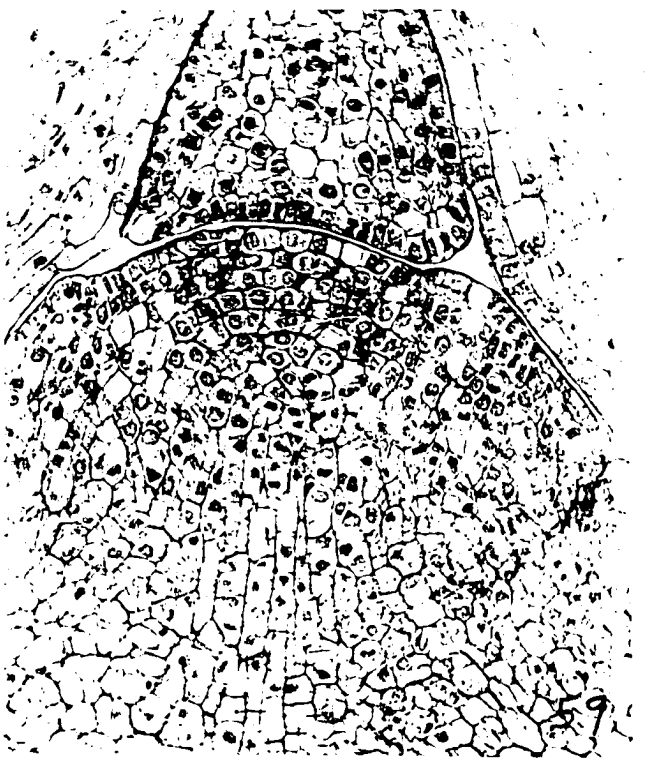
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in width and a decrease of 7 μ in height, respectively. The increase in the dimensions of the meristem appears to be due to the establishment of a third, and possibly a fourth, tunica layer and an enlargement of zone 5. Figures 49g and h are diagramatic representations of the shoot apex as it appears in side and face views in plants of this size.

Apparently, the change from a 2-layered tunica to one with 3 or 4 layers occurs at a stage in development in which the size of the stem is between 2.5 mm (42 weeks) and 6 mm in diameter. As development of the apex progresses, stratifications extend to deeper cell layers, resulting in a 3- or 4-layered tunica and a more deeply situated corpus.

Zonation within the apex is conspicuous (figs. 56, 58). The number of tunica layers appears to vary from 3 to 4 and the fourth layer is more conspicuous when the apex is observed in face view (fig. 58). No periclinal divisions have been observed in T-1, with the exception of the one in figure 59. They do occur occasionally however in T-2, T-3, and T-4, especially during leaf initiation. Divisions in diagonal planes also occur, even in T-1. In most of the longisections the pro-meristem, consisting of tunica and corpus, is quite distinct. Laterally, and at the base of the promeristem, cell divisions are more numerous and produce a cambium-like zone.

The flank meristem is well developed in apices of this size and usually consists of 4 layers of cells. It

originates from the tunica in apices with 4 tunica layers, but where there are only 3 surface layers the fourth layer apparently is derived from the outer corpus. This meristem, as was pointed out in discussing the early shoot apex, gives rise to the epidermal system of the shoot and contributes cells to the internal tissues of the developing leaf. Periclinal divisions in the second, third, and fourth layers bring about the formation of leaf buttresses (figs. 57, 59).

The outline of the apical dome varies somewhat with the stage of the plastochron. Measurements for comparative purposes were made on meristems at maximal area (figs. 56, 58). In figure 59, the apex is near minimum area, and the apical dome is considerably lower than at maximum area.

Another change in the apex in plants of this size concerns the outline as observed in transectional views. In figure 64, a transectional view 16 μ below the summit of the apical dome of a plant with a stem 7 mm in diameter and 7 mm long, the meristem is elliptical in outline; in a transection of a stem 10 x 10 mm in diameter and length, (fig. 65), it is almost circular. Apparently at this stage of development, the tendency toward this change is not widespread since with most of the longisections observed, it was not difficult to identify the two types of views. In side views (figs. 56, 57) the apex is more or less conical, whereas in face view (fig. 58) it is broad and dome-like.

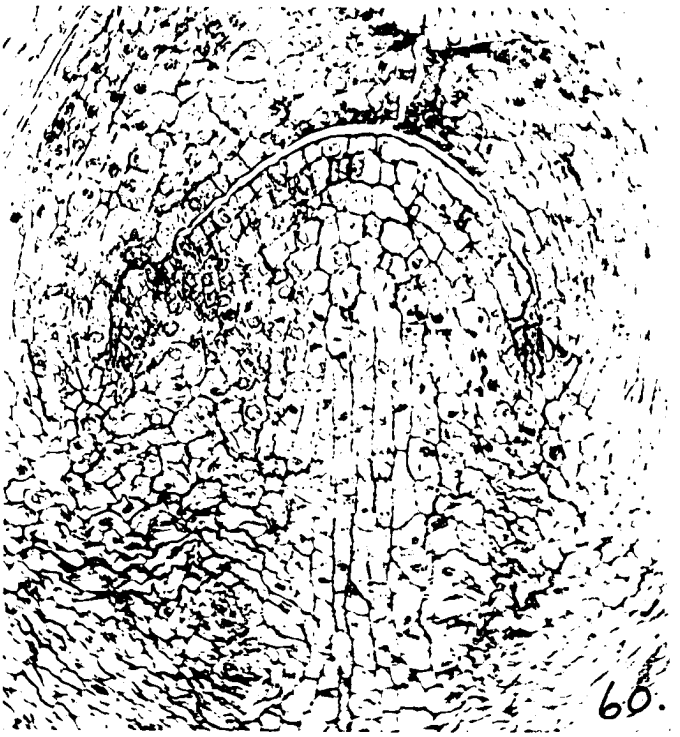
The meristems of stems 15 to 25 mm in diameter and

length were examined next. Stems from plants of this size (fig. 7f) are usually ovoid or ellipsoidal (figs. 8a, b); some however were of the shape shown in figure 8c. As observed in the field, such specimens are generally smaller than the one in figure 1, and the axis is almost always subterranean.

Before examining median longisections, transections of the apex were made (figs. 66, 67) and studied in order to ascertain if the meristem could be expected to show differences in side and face views. In figure 66 the outline of the apex is almost elliptical, but not as much so as the one in figure 67. Seemingly the form of the shoot apex varies in plants with stems 6 to 25 mm in diameter. In seedlings, where the leaves are distichous, the apex always appears elliptical in transection except near its base, and side and face views are conspicuously different. In plants with stems 6 mm or more in diameter however the arrangement of the leaves is no longer distichous. Thus the outlines of the apices in figures 64 through 66 might be due to the confining effect of surrounding leaves. The apices in figures 60 and 61 resemble side and face views of smaller plants (figs. 56, 58). They differ in the width of the rib meristem and its derivatives, which seems to indicate that they do represent different views of similar meristems. An examination of several apices from plants of this size indicates that this unsymmetrical form, characteristic of

Figure 60. A median longisection of the apical meristem from a plant with a stem 15 mm wide and 20 mm long, as observed in side view. Note the 4-layered tunica and the increase in the size of zone 5. x228.

Figure 61. A median longisection of the apical meristem of a plant with a stem 25 mm wide and 25 mm long, as observed in face view. The 5 tunica layers are clearly evident in this section. Note the stratification of the upper layer of the corpus. x226.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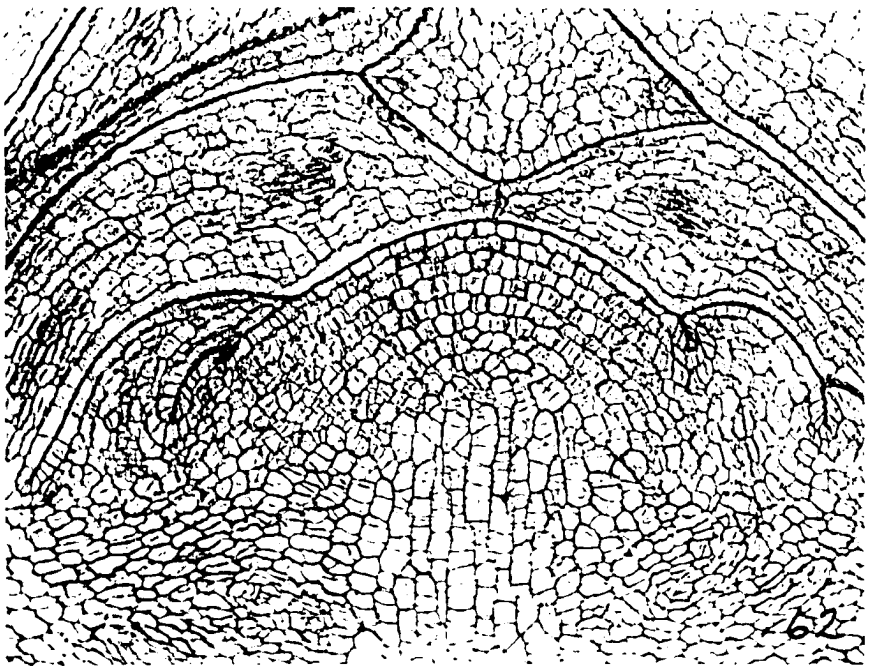
61.

Figures 62 and 63 represent median longisections of the apical meristems of medium-sized plants. It is doubtful if any distinction can be made between side and face views at this stage of development, although they might be found if enough apices were examined. In the present study it is assumed that at this stage the distinction no longer exists.

Figure 62. The meristem shown here is probably at maximum area. Note the 5 or possibly 6 tunica layers and the numerous divisions in the flank meristem. The origin and increased development of zone 5 is clearly evident. x176.

Figure 63. This apex is approaching minimum area due to the formation of leaf buttresses. Note the diagonal walls in the tunica and the periclinal divisions below the leaf buttresses. Zone 5 is well shown in this section. x176.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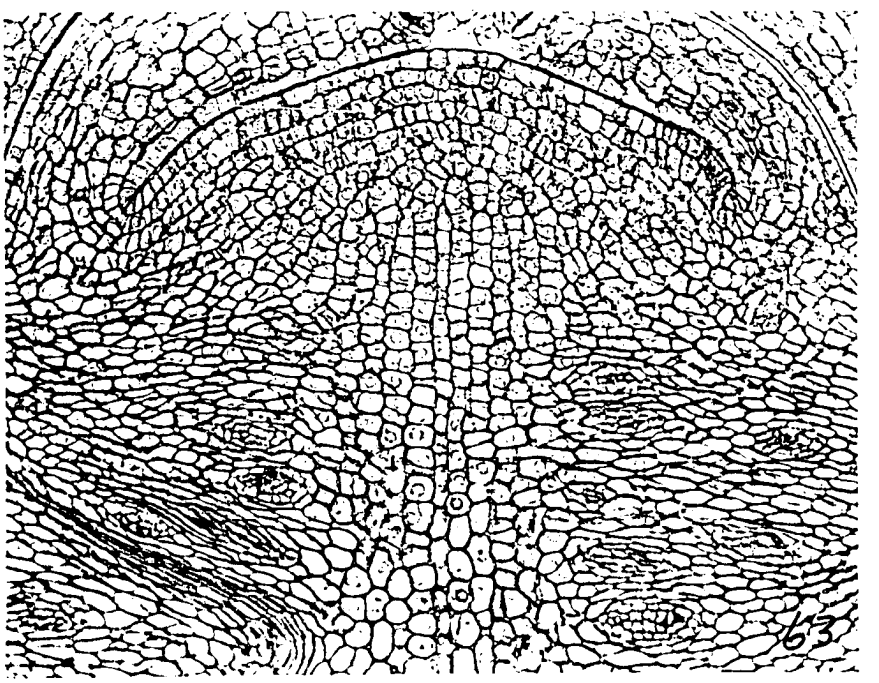
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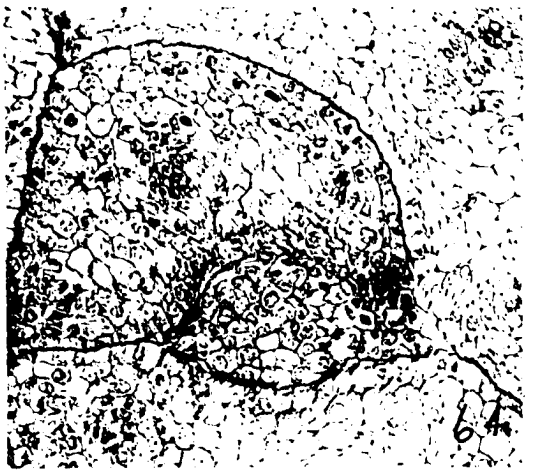
Figure 64. A transection 16 μ below the summit of the apical dome from a plant with a stem 7 mm wide and 7 mm long. Note the elliptical outline of the apex at this level. The outline of the cells of T-2 are evident in the center, surrounded by cells of T-1. x226.

Figure 65. A transection 24 μ below the summit of the apical dome from a plant with a stem 10 mm wide and 10 mm long. The meristem at this level is almost circular in outline. In the center are cells of T-2, while at the periphery cells of T-1 are visible. x226.

Figure 66. A transection 24 μ below the summit of the apical dome of a plant with a stem 15 mm wide and 20 mm long. The outline of this apex is not as circular as the one in figure 65, and not as elliptical as the one in figure 64. Apparently, in stems 10 to 20 mm in diameter, the outline of the apical meristem at these levels is variable, and seemingly signifies a transition from the elliptical type of apex, characteristic of seedlings, to the dome-shaped apices found in mature plants. In the center are cells of T-2, surrounded by cells of T-1. x226.

Figure 67. A transection 16 μ below the summit of the apical dome of a plant with a stem 20 mm wide and 20 mm long. Here the outline is elliptical, resembling the one in figure 64. x226.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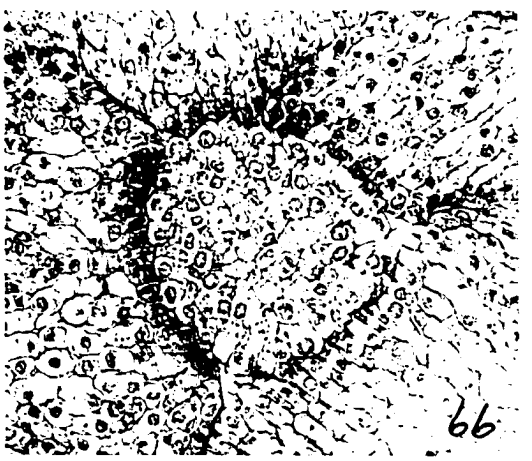
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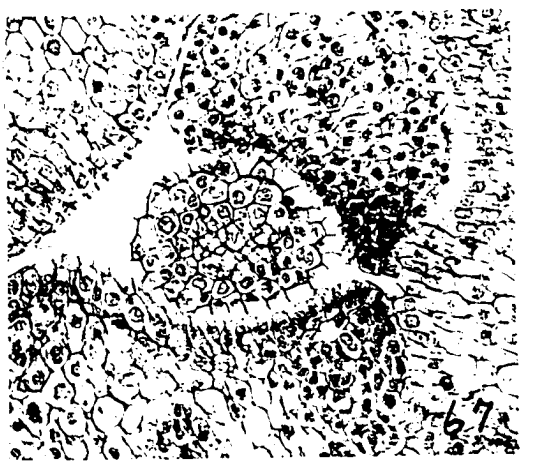

67

smaller plants, probably still exists.

The evident increases in the size of the apices in figures 60 and 61 is striking. The apex in figure 60, presumably a side view, is 231 μ wide and 112 μ high, which is 63 μ wider and 21 μ higher than the one in figure 56. The apex in figure 61, probably a face view, is 301 μ wide and 84 μ high, 105 μ wider and 14 μ higher than the corresponding view in figure 58.

A significant developmental change in apices of this size is the increase in stratification. The number of surface layers varies from 4 to 5, but 5 is the usual number. Periclinal divisions are much less frequent in the tunica layers of apices of this size than they are in the surface layers of smaller plants. The apex in figure 60 is an anomaly with respect to irregular divisions in the tunica. The corpus zone is more conspicuous in face view (fig. 61) than in side view, and its outer layer is frequently stratified.

The flank meristem usually consists of 5 layers, which originate from the 5-layered tunica, but in apices which have only 4 surface layers the meristem likewise appears to have only 4. Figures 50i and j are diagramatic representations of the apex at this stage.

The summit of the axis of most plants with stem diameters less than 15 to 20 mm is more or less rounded or

flattened, and the shoot apex is centrally located at its tip. By the time the diameter of the stem becomes as great as 20 mm, however, the shoot apex is situated at the bottom of a depression, similar to the one in figure 69.

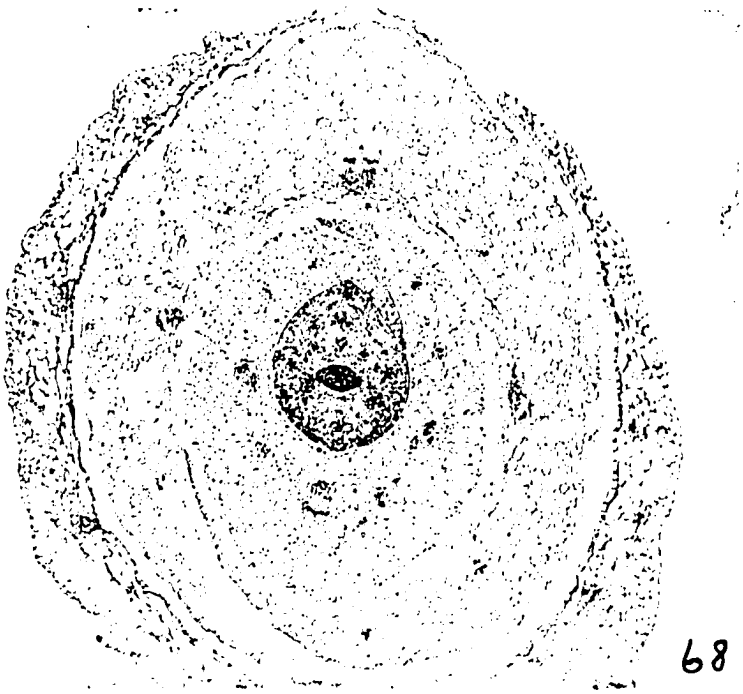
The structure and development of the apical meristem was studied next in what might be called medium-sized plants. These were about the size of the one in figure 1, but not as large as the plant in figure 2. The stems, after the leaves were removed (figs. 8e, 8f) were 20 to 30 mm in diameter at the rounded top and 40 to 50 mm at the widest point of the axis; the length was 55 to 75 mm.

Transectional views at 8 and 16 μ below the summit of the apex revealed that the outline of the meristem at these levels was elliptical in about half of the specimens examined. Below this level it becomes circular. At still lower levels the outline is probably influenced by the inner margins of the leaf primordia (fig. 70). In plants of this size the shoot apex has reached a stage of development at which a distinction between side and face views becomes impossible.

Figure 62 represents a median longisection of an apical meristem at maximum area. This apex is 357 μ wide and 105 μ high, which is 56 μ wider and 21 μ higher than the one in figure 61. It is also 126 μ wider, but 7 μ shorter, than the side view shown in figure 60. From these figures it is clear that the meristem has undergone an increase

Figure 68. A transection through the region of the apical meristem of a seedling 11 weeks old. The distichous arrangement of the leaves is clearly evident. x39.

Figure 69. Near-median longisection of the stem of a medium-sized plant showing the depression at the summit. x1/2.



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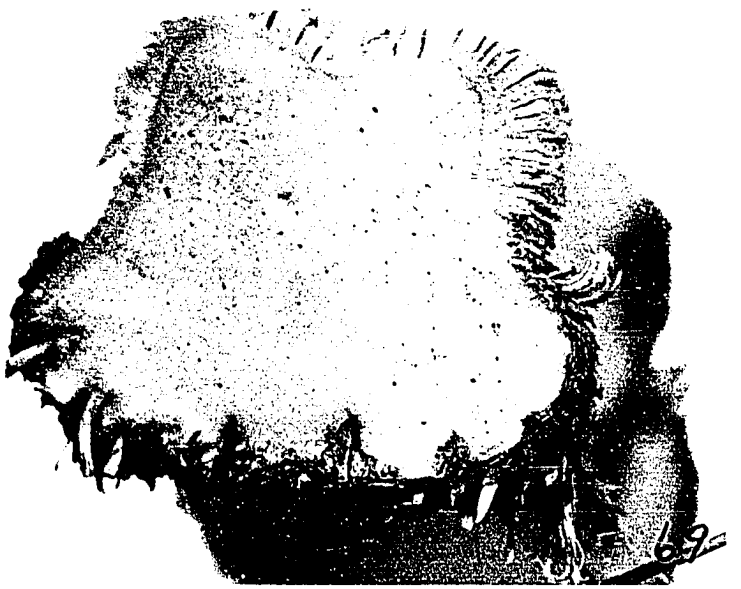

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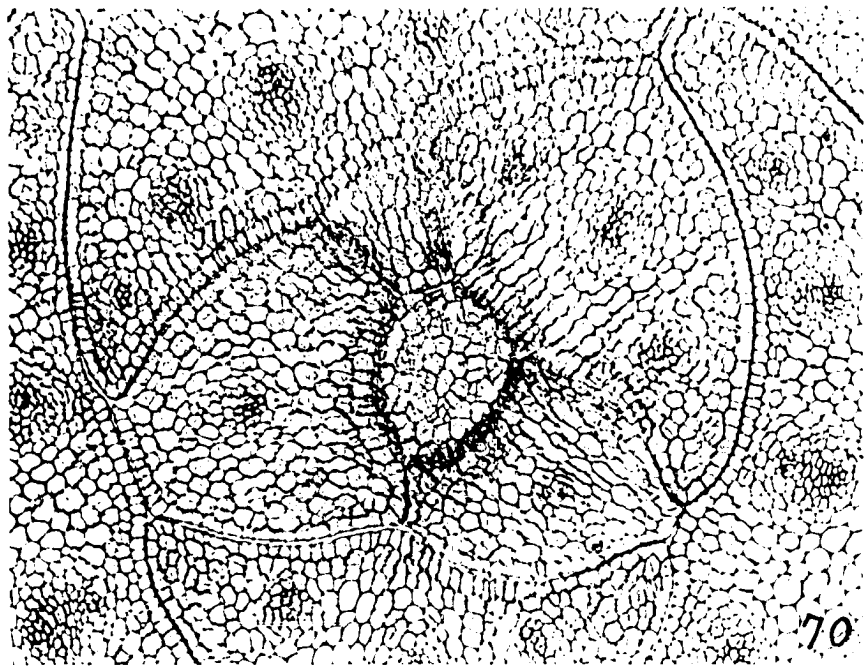
Figure 70. A transection 8 μ below the summit of the apical meristem of a medium-sized plant. The apex appears to be elliptical in outline at this level. x176.

Figure 71. A transection 32 μ below the summit of the apical dome of a medium-sized plant. This apex is probably at maximum area. Notice the circular outline of the meristem at this level. Cells of T-3 are in the center, surrounded by cells of T-1 and T-2. x176.



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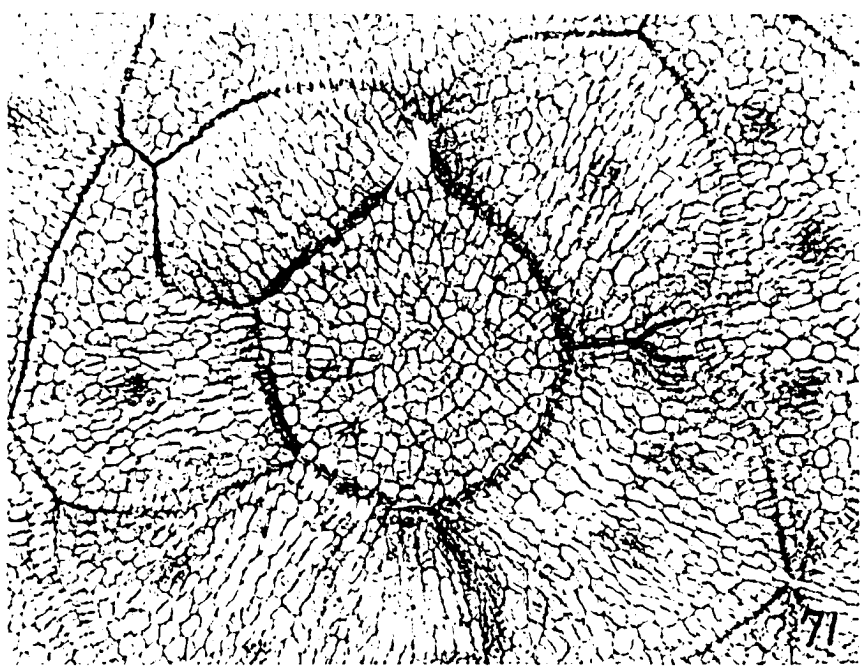


71

in width rather than in height. It has become a broad dome.

Zonation within the meristem is conspicuous. The number of tunica layers appears to vary from 5 to 6. The multilayered tunica is established gradually, as periclinal divisions in deeper layers become fewer and fewer. The tunica as a whole is more stable than in the apices of smaller plants, as evidenced by the uniformity in the size of the cells and the lack of periclines at maximum area (fig. 62). During leaf initiation however (fig. 63) numerous diagonal walls are evident, particularly in the lower layers. The corpus is more or less cup-shaped, and the upper layers are more or less stratified. The flank meristem consists of 5 or 6 layers. The numerous anticlinal divisions which characterize flank meristems may be seen in figures 62 and 63. A significant feature of the shoot apex at this stage of development is the extensive development of zone 5 and its derivatives (fig. 63). At about the level of the rib meristem the derivatives of this zone spread laterally beneath the leaves. The primary thickening meristem originates here. Cells of zone 5 divide in diverse planes. Beneath the leaves, divisions in this zone tend to become restricted to planes at right angles to the axis. Vascular bundles occur in the deeper parts of the derivatives of zone 5. Figure 50k is a diagramatic representation of the apex at this stage of development.

The shoot apex is situated at the bottom of a depression (fig. 69) which varies in depth. In stems 20 to 25 mm in diameter and length it is usually about 1 mm deep, but in larger stems (figs. 16, 17) it may be 3 mm deep.

Figures 70 through 73 are transections of the apical meristem and its derivatives at various levels. Cells of T-1 are about 12 microns high. Hence in figure 70, a transection 8 μ below the top of the dome, cells of T-1 are still visible. Figure 71 is a transection 32 μ below the tip of the meristem, and in its center are cells of T-3; further out cells of T-2 and T-1 can be recognized. Two hundred microns below the tip (fig. 72) the rib meristem appears in the center, surrounded by derivatives of the flank meristem. Four hundred and seventy-two microns down (fig. 73), derivatives of the rib meristem are larger and occupy a greater area than at higher levels.

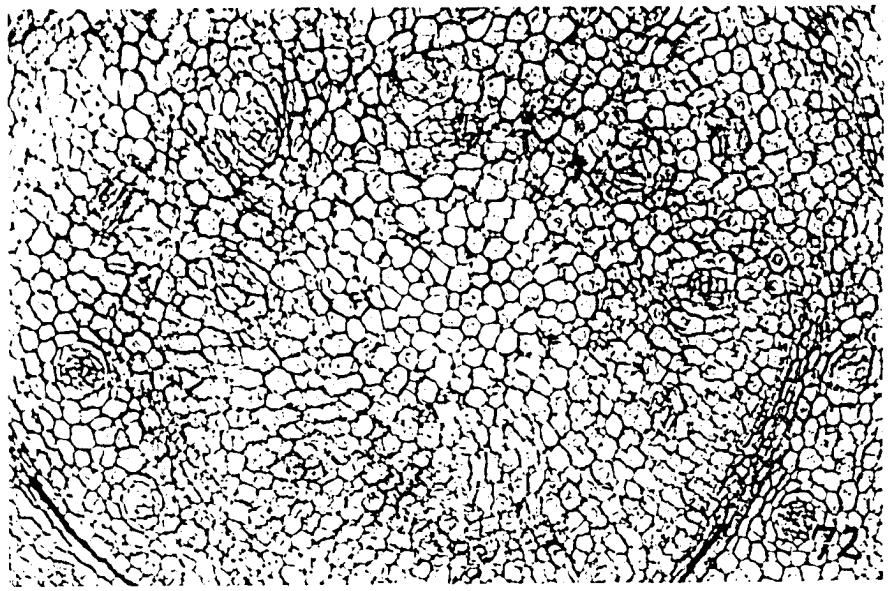
Material for the study of the fully developed shoot apex was obtained from the largest plants available. Such specimens (figs. 2, 3) have stems which often measure 4 to 6 inches in diameter at their bases (figs. 9, 16). The diameters of the rounded tips of such specimens (figs. 22, 74) varied from one and one-eighth to two inches.

The apical meristem in mature plants is a broad dome (figs. 75, 76). The apices in figures 75 and 76 and 63 μ high and 392 and 448 μ wide, respectively. The average width of these two apices is 415 μ, which is 58 μ greater

Figures 72 and 73 represent transections of the apical meristem of medium-sized plants.

Figure 72. A transection 200 μ below the summit of the apical dome. In the center are cells of the rib meristem, surrounded by cells which make up the area (zone 5) below the flank meristem. x176.

Figure 73. A transection 472 μ below the summit of the apical dome of a medium-sized plant. At this level the area formed by the derivatives of the rib meristem appear to broaden considerably, and enlargement of the cells is evident. x176.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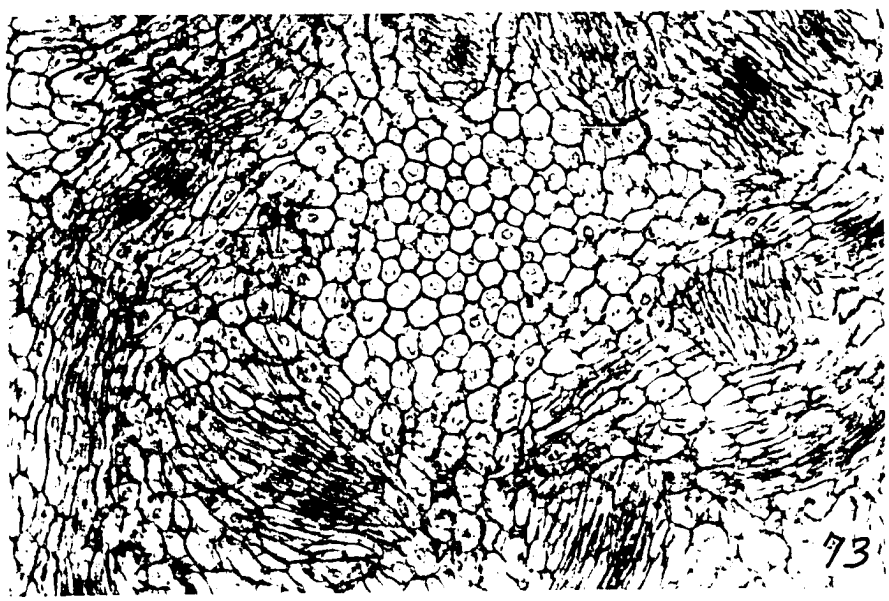
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Figure 74. Photograph of part of the stem of an adult plant. The numerous lines evident on the stem mark the places where leaves were once attached. Note the large rounded tip at the summit, and the lighter area below it, extending down to the larger part of the stem; this area, below the rounded summit, marks the places where the living leaves were attached. The rounded tip is 2 inches in diameter at its widest point.

Figure 75. A median longisection of the apical meristem of an adult plant. The 6-layered tunica and the corpus are highly stratified. Note the extreme development of zone 5. x176.

74

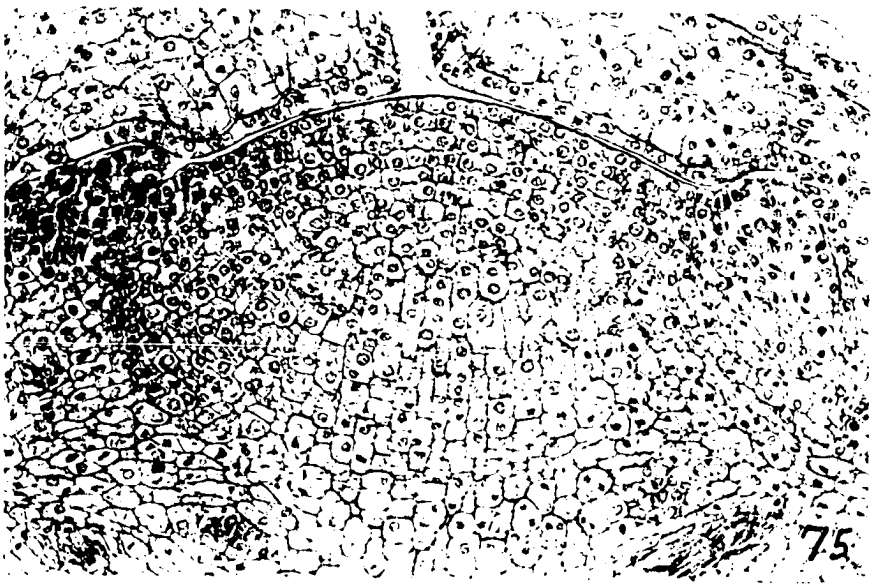

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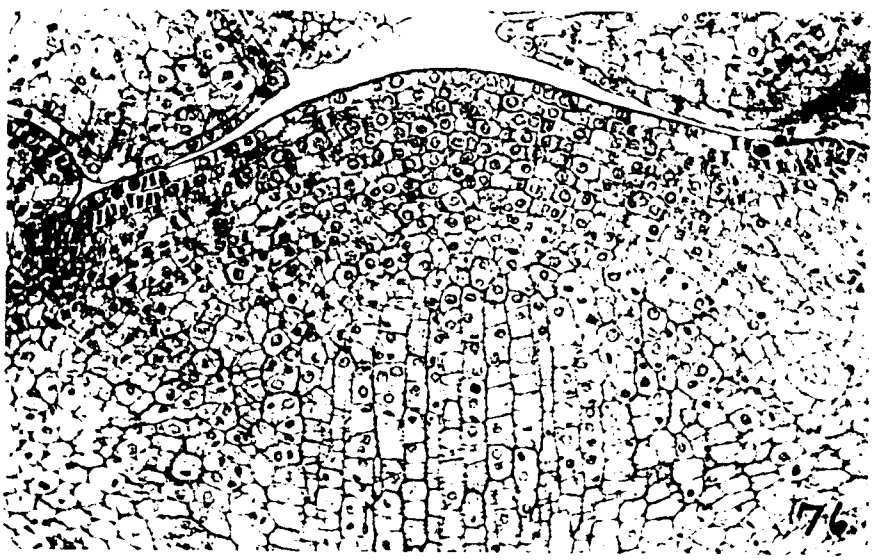
Figure 76. A median longisection of the apical meristem of an adult plant. This apex is 420μ wide, somewhat wider than the one in figure 76, and is near maximum area. Numerous divisions in diverse planes are evident in zone 5 on the left, below the leaf buttress. x176.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than the apex in figure 62; the height however is only 63μ, which is 42 μ less than the one in figure 62. On the basis of these measurements it appears that one of the final stages in ontogeny of the meristem is a broadening and flattening of the apical dome.

Zonation within the apical meristem is conspicuous for all zones except the corpus, the upper limit of which is indistinct in some specimens. There are 6, or possibly 7, tunica layers, the cells of which are more or less uniform in size, vacuolation, and staining. No periclinal divisions have been observed in T-1 and only a few in the lower tunica layers (fig. 76). Individual cells of the tunica are somewhat smaller than the corresponding cells in apices of smaller plants. The corpus is much broader and its upper boundary is indistinct. The individual cells of this zone are somewhat larger than tunica cells, except in the outer corpus, and stain somewhat less intensely. The peripheral zone, or flank meristem, consists of from 6 to 7 layers, depending upon the number of tunica layers.

The rib meristem and its immediate derivatives are conspicuous features of the fully developed apical meristem, and in general are wider, as observed in median longisection, than those in less mature meristems (compare figures 62 and 63 with figure 75 and 76). Cells of this zone are highly vacuolated and hence stain lightly. Derivatives of the rib meristem elongate parallel to the axis. A few cell divisions

still occur, often in oblique planes. The derivatives of the rib meristem form the central part of the axis. Zone 5 is highly developed in the mature apical meristem and is visible as a large group of irregularly shaped cells outside the rib meristem. Zone 5 originates at the sides of the corpus just below the flank meristem. Figure 50 1 is a diagramatic representation of the fully developed apical meristem.

Initiation and Early Development of the Leaf

Leaf initiation begins with periclinal divisions in the flank meristem. The precise layer in which this occurs varies with the stage of development of the shoot apex. Thus in seedlings (fig. 54) it occurs in the third layer, while in medium-sized plants it seems to begin in the fifth layer (fig. 63). Once the divisions have begun, they spread outwardly until they reach the second layer where they usually stop. Occasionally however they continue into the first layer (figs. 59, 63). Zone 5 appears to become more and more involved in leaf initiation as the shoot apex grows larger (figs. 59, 76).

As the result of these early divisions a slight bulge or "foliar buttress" (Foster, 1936) occurs on the side of the apex. In seedlings the buttresses are usually 180 degrees apart. When apices of seedlings undergoing leaf initiation are observed in side view (figs. 54, 88), the buttress appears on the side of the apex directly opposite the

youngest leaf. In older plants, in which the leaf arrangement is alternate, the buttresses are arranged in a spiral. Thus, in longisection (fig. 63), two buttresses at different stages of development often appear in the same section. As tne leaf buttress continues to develop the divisions gradually extend laterally. Eventually a leaf primordium arises as a protuberance on the foliar buttress. In seedlings this protuberance assumes the form shown in figures 42 and 43, and is very close to the center of the shoot apex. In older plants its distance from the center varies, depending upon the size of the apex (compare apices in figures 57, 62, 75). In seedlings, the lateral extension of the divisions mentioned above eventually causes the primordium to encircle the shoot apex, somewhat like a collar (fig. 53). The central part of the primordium grows faster than the lateral parts thus causing the latter to slope downward on each side. In older plants however only a part of the apex is encircled (figs. 66, 70). The structure of the seedling primordium is similar to that of a grass leaf primordium. Esau (1953) in discussing the collar-like primordium in the grass apex, refers to the upward growth of the median part of the collar as "apical growth" and the upward growth of the margin as "marginal growth". Esau (1953) further states that "the combined apical and marginal growth of the grass leaf lasts a short time, but intercalary growth at the base of the primordium continues the process of elongation." A similar

situation appears to exist in the seedling of Dasylirion.

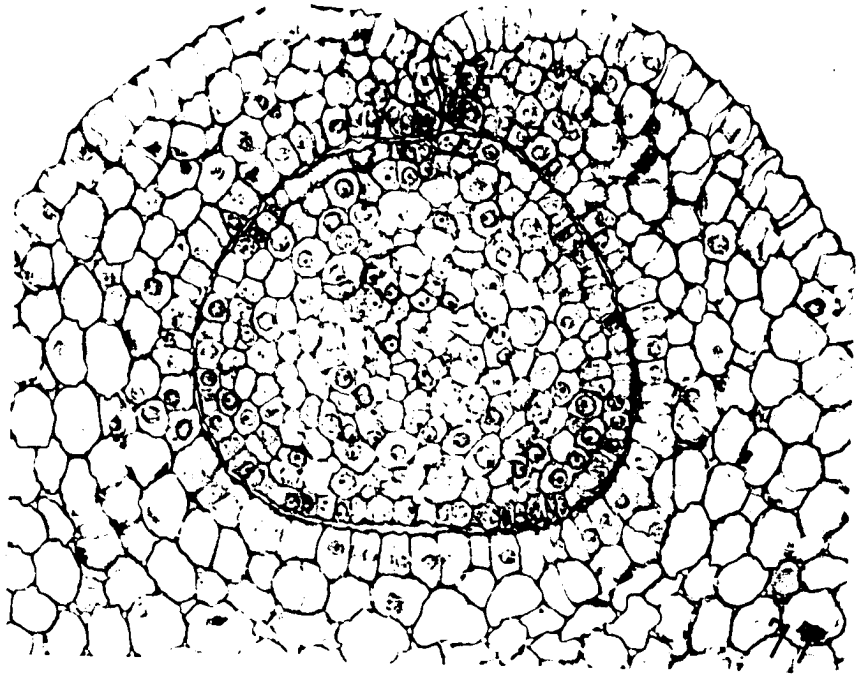
Figure 42 represents a stage in the development of the primordium in which the intercalary meristem is evident at the base (compare this stage with the one in figure 41). The anticlinal walls are more obvious at the upper part of the base than at the lower part. Derivatives from the 3 layers of the flank meristem can be seen in the primordium. They appear to maintain their identity almost to the tip, where divisions in various planes have occurred. On the abaxial side of the primordium, the second and third layers have undergone periclinal divisions, thus contributing to the interior of the primordium. The primordium at this stage extends 28 μ above the higest point of the apex; at its base procambium has begun to form. When the apex of the primordium is 42 μ above the upper limit of the meristem (fig. 43) the procambium shows further development and extends about half the length of the primordium. The second and third layers of the primordium now lose their identity, especially near the tip, and development inside the primordium is by general cell division, although intercalary growth at the base continues. The procambium continues to develop acropetally and eventually reaches the summit of the primordium (fig. 44), which by this time is 112 μ above the highest point of the shoot apex.

Figure 29 represents a median longisection of the primordium of the first leaf of a seedling 3 mm long. In

figure 77, which is a transection of a seedling of this size through the region of the cotyledonary slit, the developing procambium is visible in the center, surrounded by cells more or less loosely arranged. Divisions in the subepidermal layers have occurred in various planes. As the primordium continues elongation the loose arrangement of the cells (fig. 43) is gradually replaced by a more orderly one. The beginning of this is evident in figure 44, while a later stage is shown on the right in figure 54. For some time after the development of the median procambial strand in the center of the primordium, the young leaf enlarges radially by divisions in the second and third layers. As the leaf continues to enlarge in this manner, and to elongate by means of the intercalary meristem at its base, it gradually reaches a size (fig. 78) at which 2 lateral procambial strands are formed. From this point on, thickening of the leaf occurs by means of adaxial and abaxial meristems. The abaxial meristem appears to be the most active and its activity continues for a longer period of time. In figure 79, the abaxial meristem is labelled "a" and the adaxial "b". In figure 82 the abaxial meristem is indicated by the arrow. Marginal growth occurs by means of initials which become most active after the 2 additional procambial strands begin to form. The location of these initials with respect to the 2 additional procambial strands is indicated by the arrow in figure 78. As the leaf expands in width more procambium is formed and the area

Figure 77. Transection through the region of the cotyledonary slit of the young leaf of an emergent seedling 3mm long. Note the procambium in the center and the irregular outlines of the cells making up the second layer. The young leaf is surrounded by the cotyledonary sheath. x228.

Figure 78. A transection 56 μ below the summit of the apical dome of a plant 15 weeks old. The arrow indicates the location of one of the marginal meristems. x228.



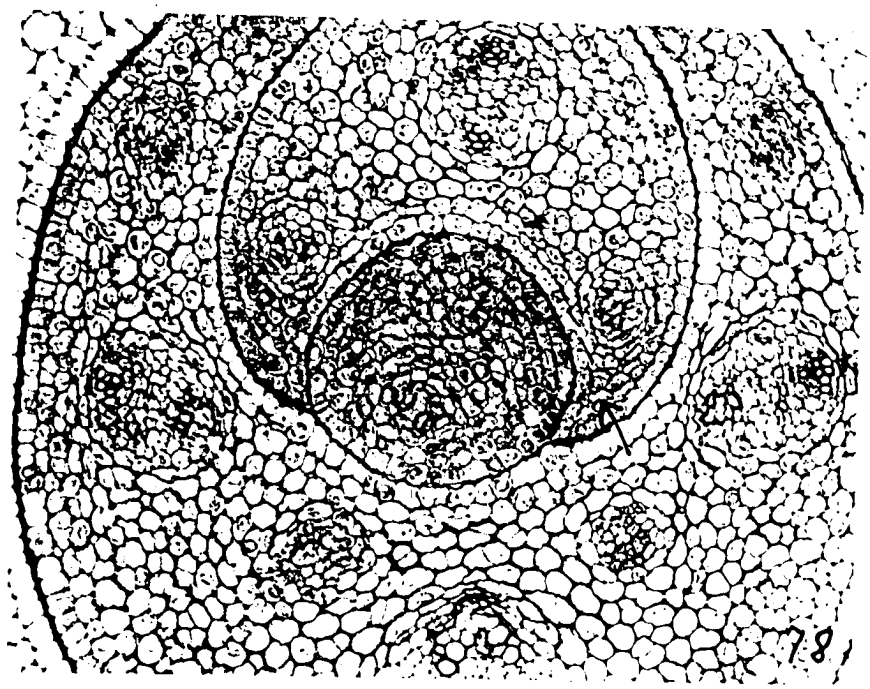

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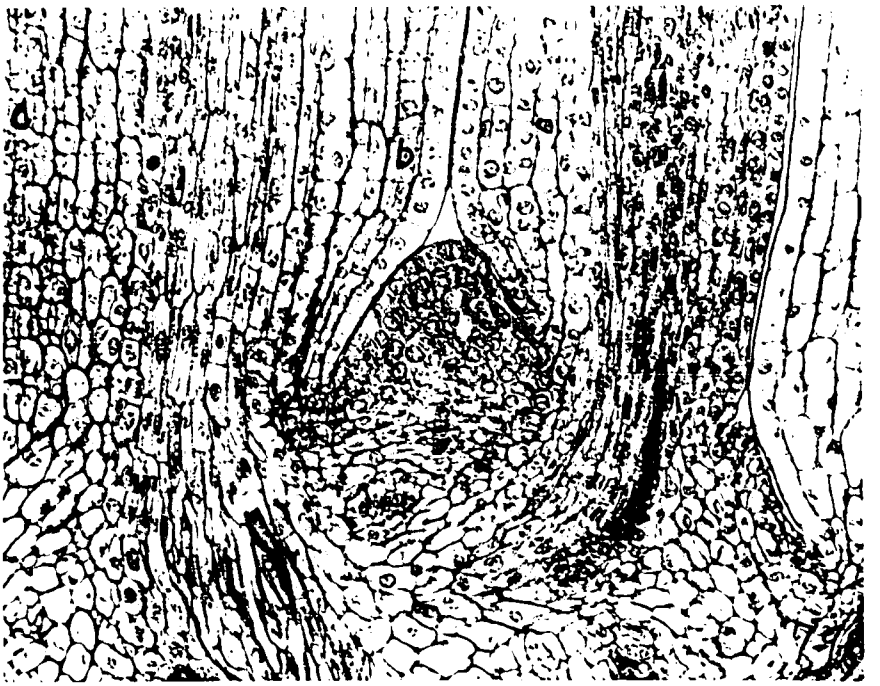
Figure 79. Near-median longisection of the apical region of a seedling 12 weeks old. Note the numerous divisions which have occurred in the areas labelled "a" and "b", in the young leaf to the left. x228.

Figure 80. A transection 8 μ below the base of the apical meristem of a seedling 15 weeks old. Note the numerous divisions in diverse planes which have occurred in the marginal meristem, as indicated by the arrow. x228.


a
b

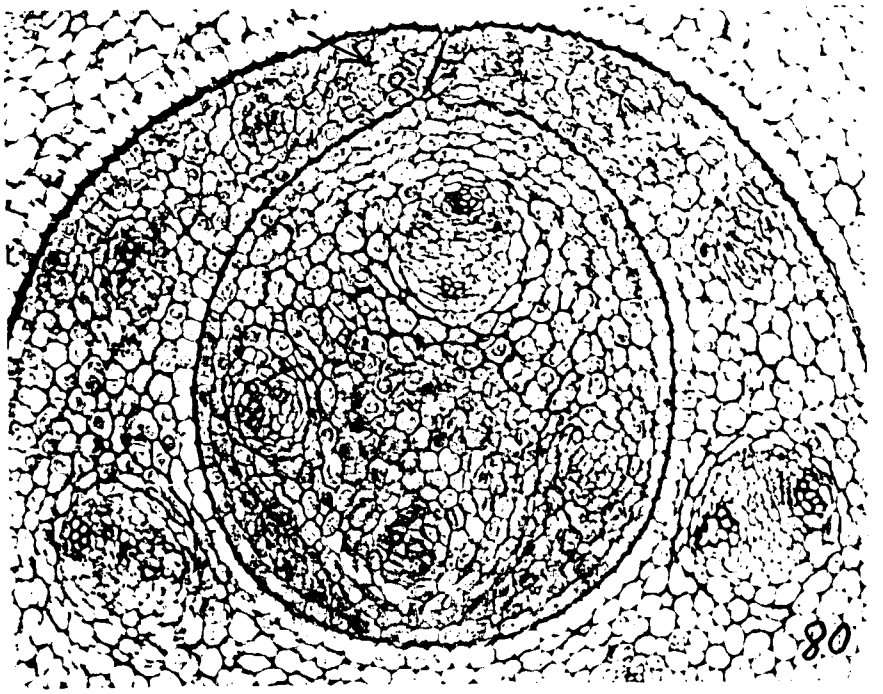

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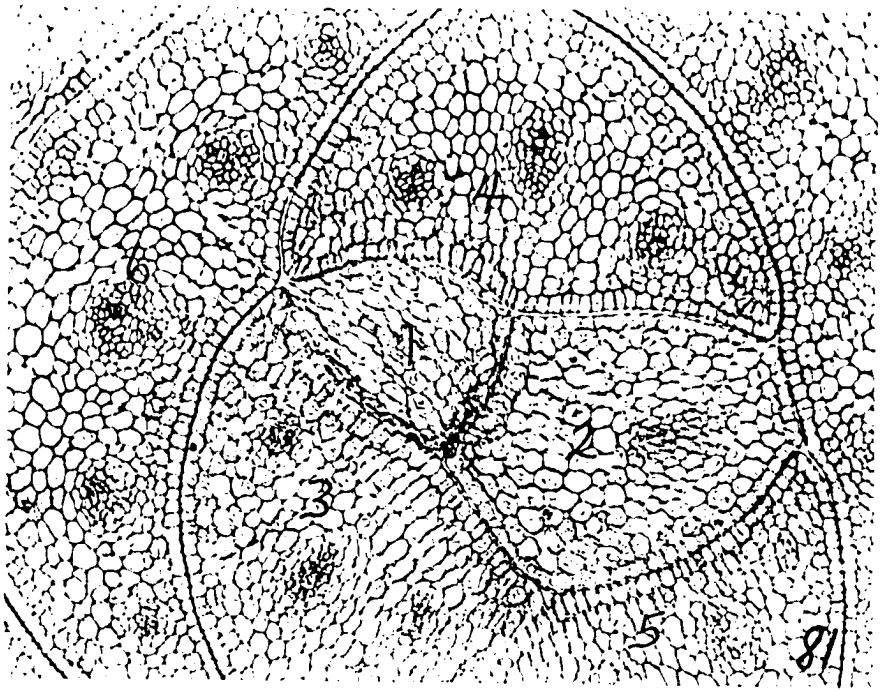
Figure 81. A transection 24 μ above the summit of the apical dome of a medium-sized plant. By going from 1 to 6, five leaves are passed and the stem is encircled twice. Leaf 6 is about on the same vertical line as leaf 1. Such an arrangement of leaves is known as two-fifths phyllotaxis. Numerous periclinal divisions have occurred in the subepidermal layers of the leaves. Note periclinal divisions in the abaxial meristem of leaf 6, and in the adaxial meristem of leaf 4. x228.

Figure 82. A transection through the summit of the apical dome of a medium-sized plant. The arrow points to the abaxial meristem of a leaf. x228.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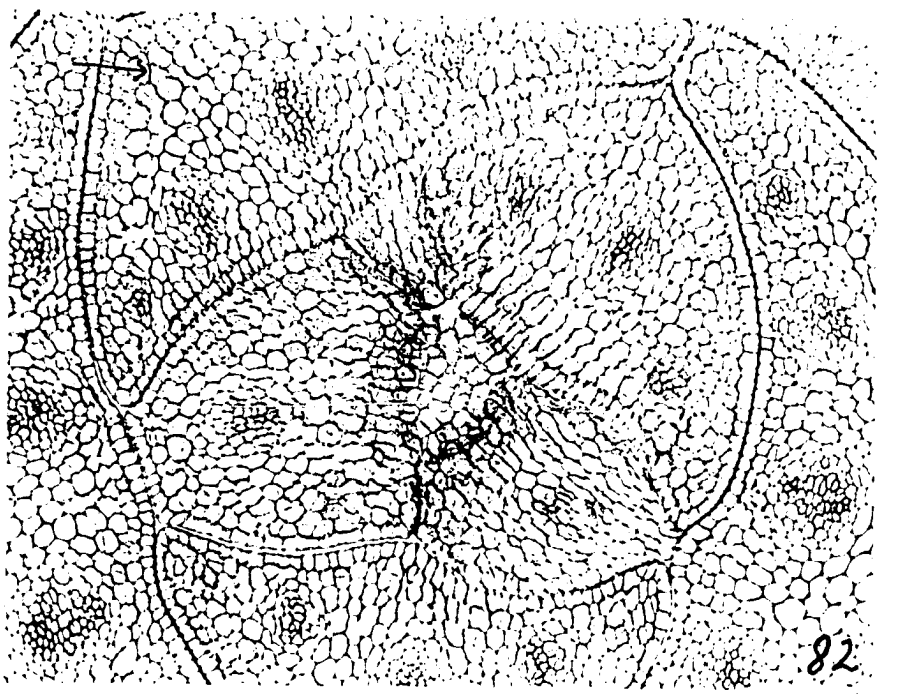
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occupied by the initials becomes long and narrow (fig. 80).

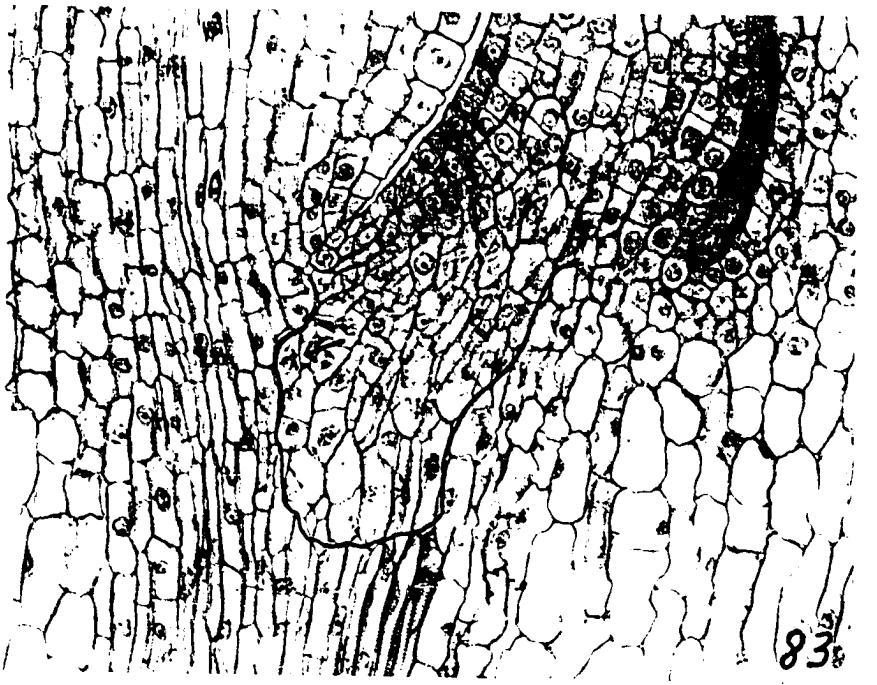
By the time the stem is 6 mm in diameter the leaf arrangement usually becomes alternate. It seems probable that most plants with stems 6 mm or larger in diameter have what is called 2/5 phyllotaxis. According to Esau (1953), "in a shoot having a 2/5 phyllotaxis one must pass 5 leaves along a continuous helix and encircle the stem twice before coming to a leaf inserted approximately on the same vertical line as the one with which the count was started." Figure 81 represents a transection 24 μ above the summit of the apical dome of a medium-sized plant, and illustrates how the above principle can be applied to this plant in determining the phyllotaxis. It seems probable that transition forms, representing some intermediate leaf arrangement, might be found if a larger number of specimens could be examined.

Structure and Development of the Primary and Secondary Thickening Meristems

The embryonic stem is visible in the embryo as a small cluster of irregularly arranged cells situated below the apical meristem (fig. 24h). After germination of the seed has begun and the emergent seedling has reached a length of 2 to 3 mm, the stem appears in near-median longisection as shown in figures 28, 29, and 83. The arrangement of the cells making up the axis is more regular than in the embryo and their long axes are directed vertically. The area

Figure 83. Near-median longisection of the apical meristem and axis of an emergent seedling two and one-half mm long. Note the enlarged cells and the conspicuous planes of divisions. The arrow points to a cell in metaphase. x228.

Figure 84. A transection 156 μ below the base of the leaf of an emergent seedling 3 mm long. Note the periclinal divisions in some of the parenchyma cells just outside the stele. x228.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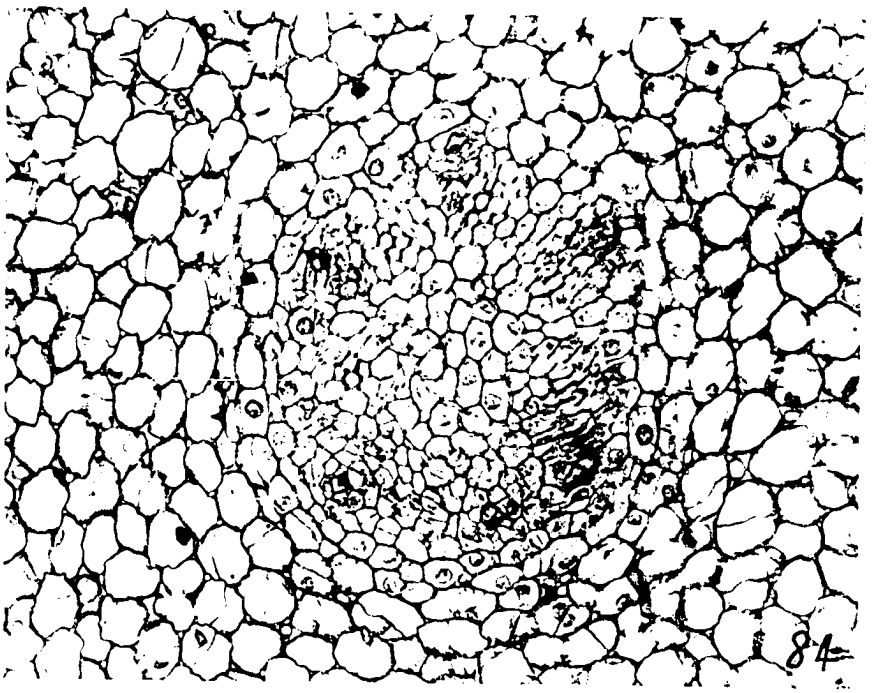
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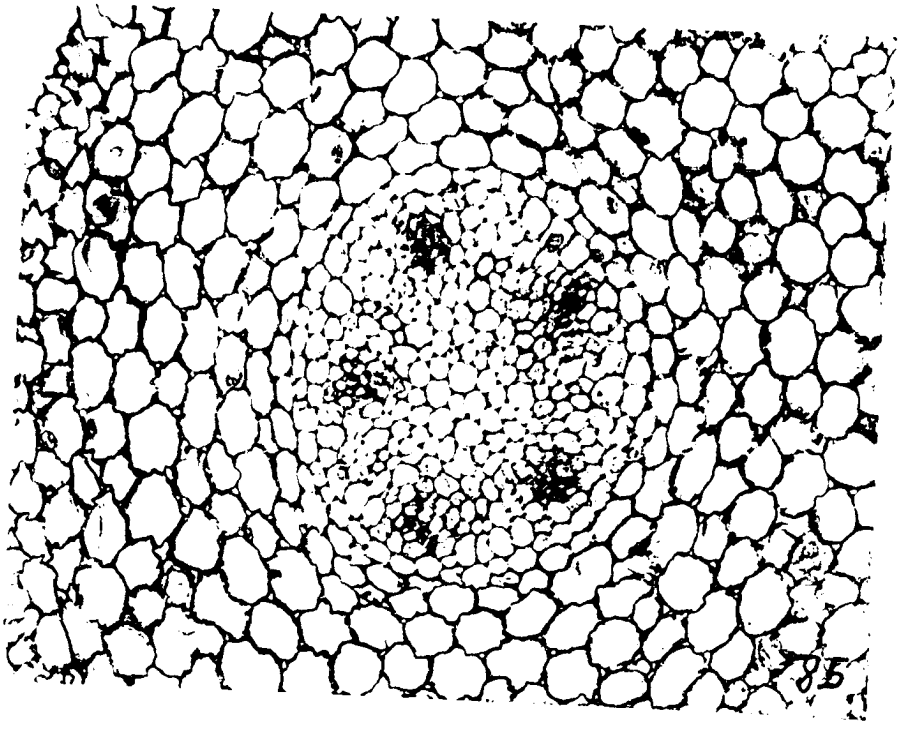
occupied by the developing stem at this age is indicated in figure 83 by the heavy line. The increase in the size of the young axis (compare with figure 24h) results from the enlargement and divisions of the cells originally present in the embryonic axis. In figure 29d, which represents a somewhat later stage of development, the axis appears larger and an increase in the number of cells is evident. Figure 84 is a transectional view of the axis 156 μ below the base of the leaf, and represents the early stele. In its center are cells derived from the embryonic axis, surrounded by procambial strands. Just outside the stele, a few parenchyma cells have undergone periclinal divisions, which apparently contribute to the development of the cortex of the stem. At 322 μ below the base of the leaf (fig. 27) these divisions are no longer visible (fig. 85).

The axis of an emergent seedling 12 mm long appears in median longisection as a "V" shaped area just below the apical meristem (figs. 35, 36). As compared with the corresponding area in figure 29, the young axis shows a further increase in size. In a transection 128 μ below the base of the leaf (fig. 86) the axis is cut at about the level indicated by the arrow in figure 34. In the meristematic parenchyma just outside the stele (fig. 86), periclinal and anticlinal divisions occur. Although this activity is a continuation of that observed in the cortex of the 3-mm seedling, it is much more extensive. Similar divisions

Figure 85. A transection 322 μ below the base of the leaf of an emergent seedling 3 mm long. At about this level the divisions in the parenchyma cells just outside the stele appear to cease. x228.

Figure 86. A transection 128 μ below the base of the leaf of an emergent seedling 12 mm long. Note the increase in the number of divisions in the parenchyma just outside the stele in this section as compared with those in figure 84. Larger cells further out in the cortex are also dividing in diverse planes. x228.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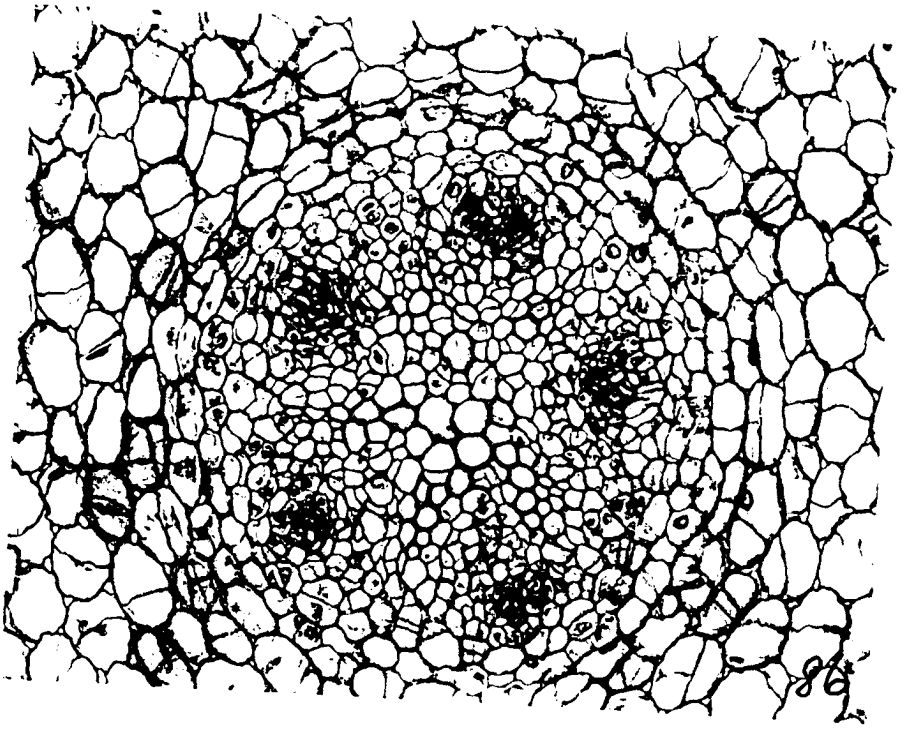
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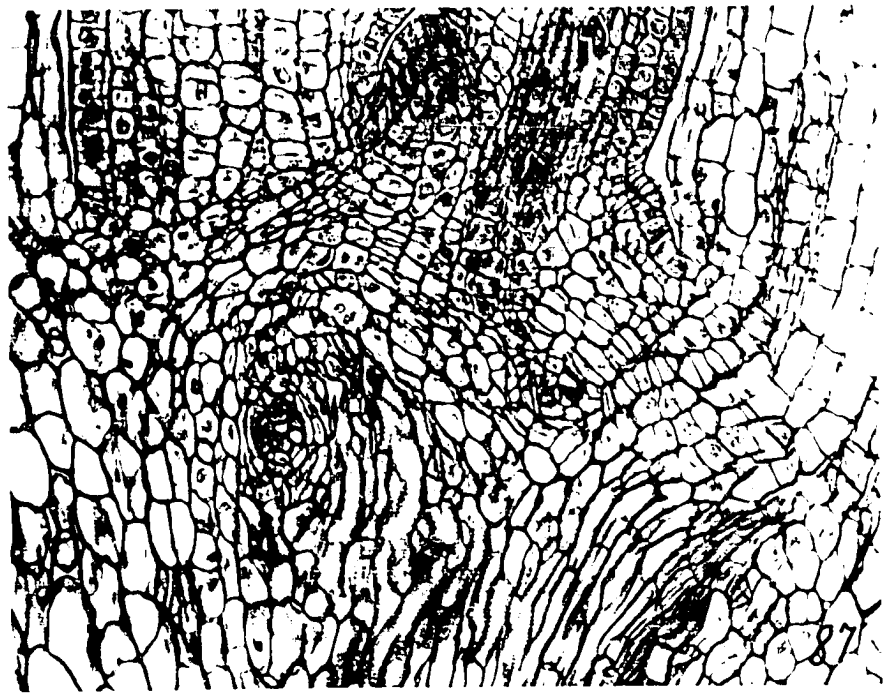
may be observed in isolated cells further out in the cortex. They contribute to the formation of the early cortex of the axis and represent the area in which the primary thickening meristem eventually arises.

In a 2-weeks' old seedling (fig. 20c), the meristematic area outside the stele is visible as a continuous zone on each side of the stele from points just below the shoot apex to the lower limit of the stem (fig. 87). This zone is the primary thickening meristem, which originates when periclinal divisions occur in the derivatives of zone 5. The upper arrow in figure 88 points to the origin of the meristem in that section. However, it is difficult to determine exactly when the primary thickening meristem originates at lower levels. It is possible that the isolated periclinal divisions which were first observed in the 3-mm seedling (fig. 84), represent the origin of the meristem at that level. It appears more likely however that the meristem originates only after the zone of periclinally dividing cells becomes more continuous (fig. 86). It is difficult to decide just when the meristematic parenchyma outside the stele becomes the primary thickening meristem, especially since both appear to contribute cells to the same part of the cortex.

In figure 88, which is a median longisection of an axis of a 5-weeks' old seedling, the outline of the thickening meristem is that of an inverted cone. As was stated

Figure 87. Median longisection of the apical meristem and part of the axis of a seedling 2 weeks old. The primary thickening meristem is visible on each side of the axis but appears particularly well developed on the right side. x228.

Figure 88. Median longisection of the apical meristem and axis of a seedling 5 weeks old. The derivatives of the rib meristem form the core of the axis, at each side of which, especially at the summit of the stem, some of the derivatives of zone 5 are added. Other derivatives of zone 5 diverge laterally and form a thin area below the primordia and young leaves. At the upper end of this thin area, the primary thickening meristem originates. The upper arrow indicates the approximate origin of the meristem. Note the intercalary meristem at the base of the leaf on the left. The primary thickening meristem increases the thickness of the axis in the areas outside and below zone 5. x150.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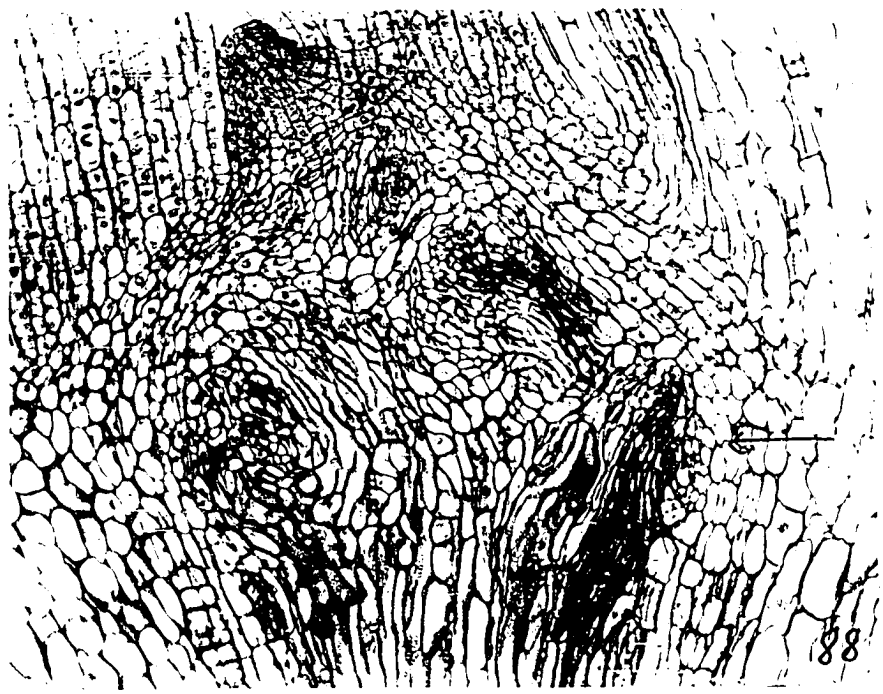
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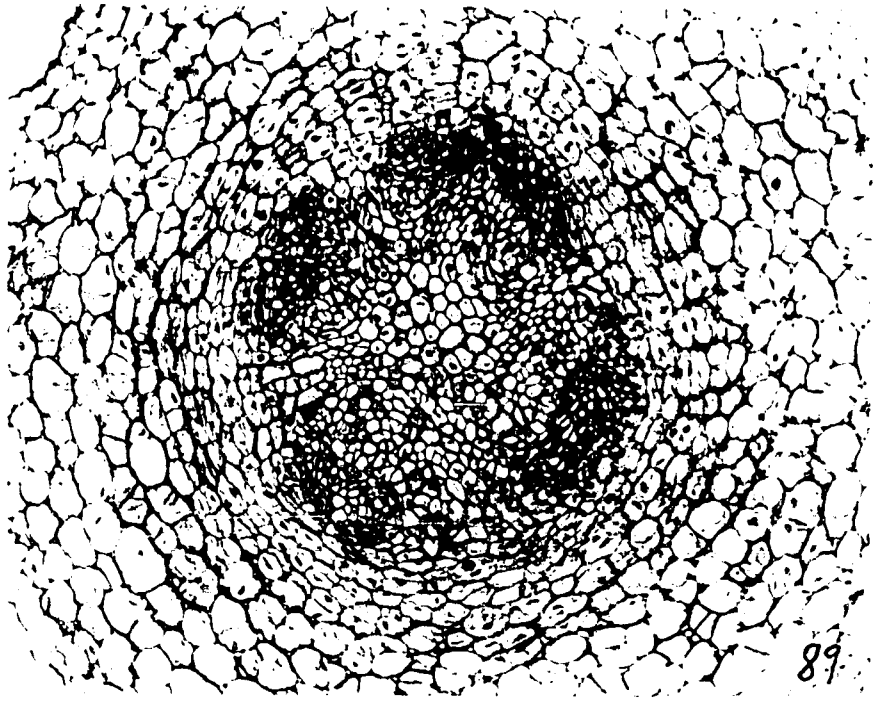
previously, the rib meristem contributes cells to the central core of the axis and zone 5 adds cells to the area lateral to the rib meristem. Most of the increase in the size of the primary body comes, however, from the activity of the thickening meristem. In the upper half of the axis, it is evident that periclinal divisions in the primary thickening meristem have produced columns of cells which appear to diverge from the axis at an angle. As more and more periclinal divisions occur, this part of the axis gradually expands upward and outward. In the lower half of the stem the same process occurs but the angle at which the cell rows diverge varies. Figure 89 is a transection 368 μ below the base of the youngest leaf; the approximate location of a section at this level is indicated by the lower arrow in figure 88. When the axis is 1 mm in diameter at its widest point (fig. 90), the primary thickening meristem appears in transection as a cambium-like zone (figs. 91, 92).

In transections of 11-weeks' old seedlings it is possible to identify very young vascular bundles just inside the thickening meristem, if the transections are made about midway between the upper and lower extremities of the axis. The appearance of these young bundles marks the beginning of secondary growth. The primary thickening meristem thus intergrades with the secondary thickening meristem in this area. As the axis continues to elongate, the region of secondary growth gradually extends upward. In large stems

Figure 89. A transection 368 μ below the base of the youngest leaf of a seedling 5 weeks old. The lower arrow in figure 88 indicates the approximate level of this section. Cells formed on the outside of the meristem contribute to the cortex of the primary body. x150.

Figure 90. Near-median longisection of part of a seedling 8 weeks old. At the two sides of this section the remains of the cotyledon are visible. Note the leaf traces. x39.



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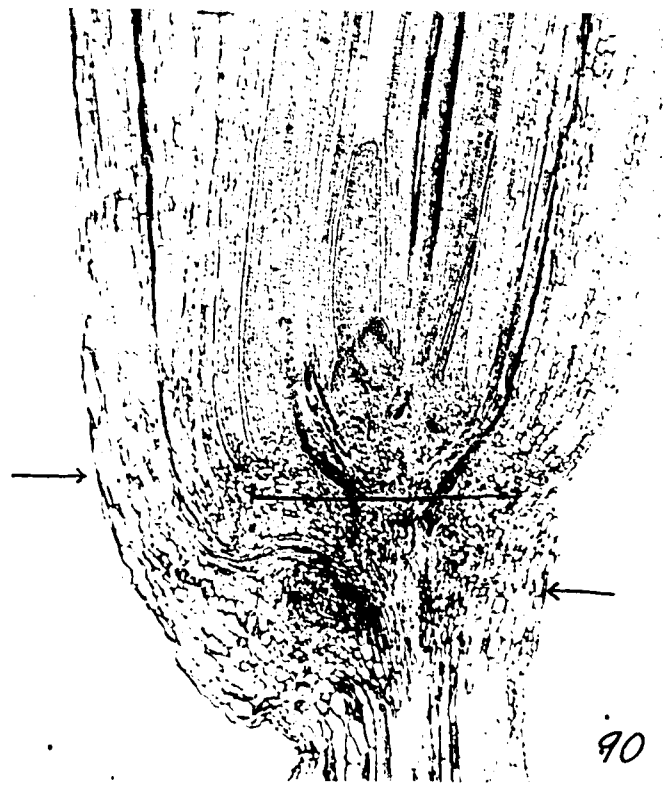

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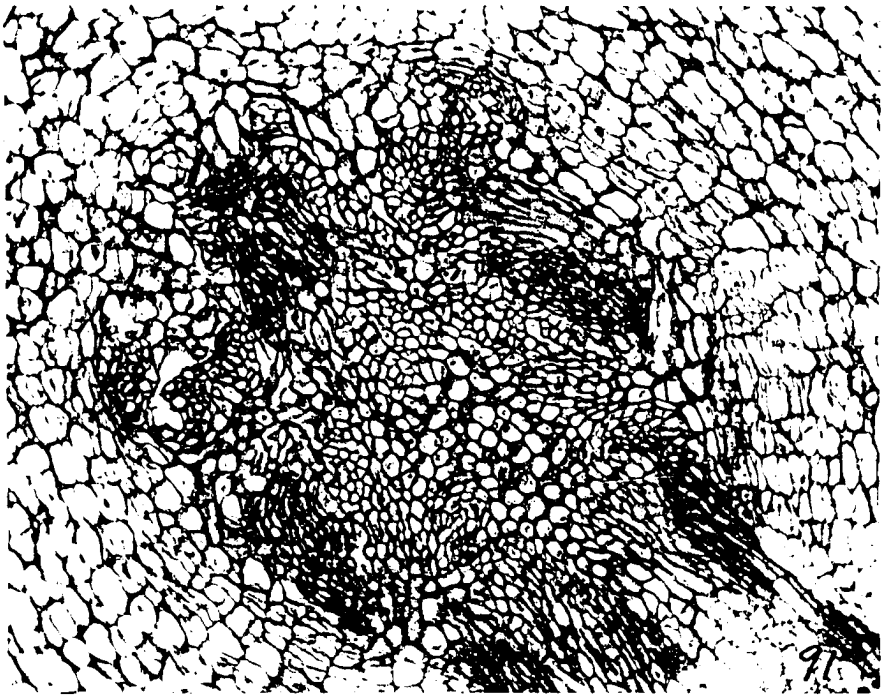
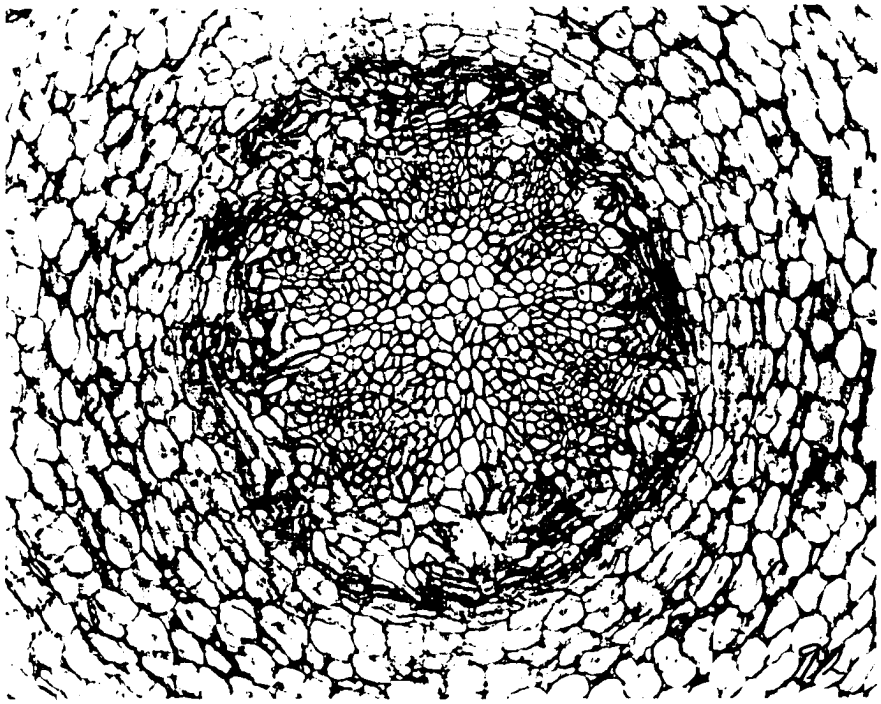
Figure 91. A transection 522 μ below the base of the youngest leaf of a seedling 10 weeks old. Note the increase in the thickness of the primary thickening meristem. At this level some of the leaf traces enter the stele in a plane almost at right angles to the axis (see lower right in this figure). The upper arrow in figure 90 indicates the level at which this section was taken. x150.

Figure 92. A transection 864 μ below the base of the youngest leaf of a seedling 10 weeks old. The lower arrow in figure 90 indicates the level of this section.





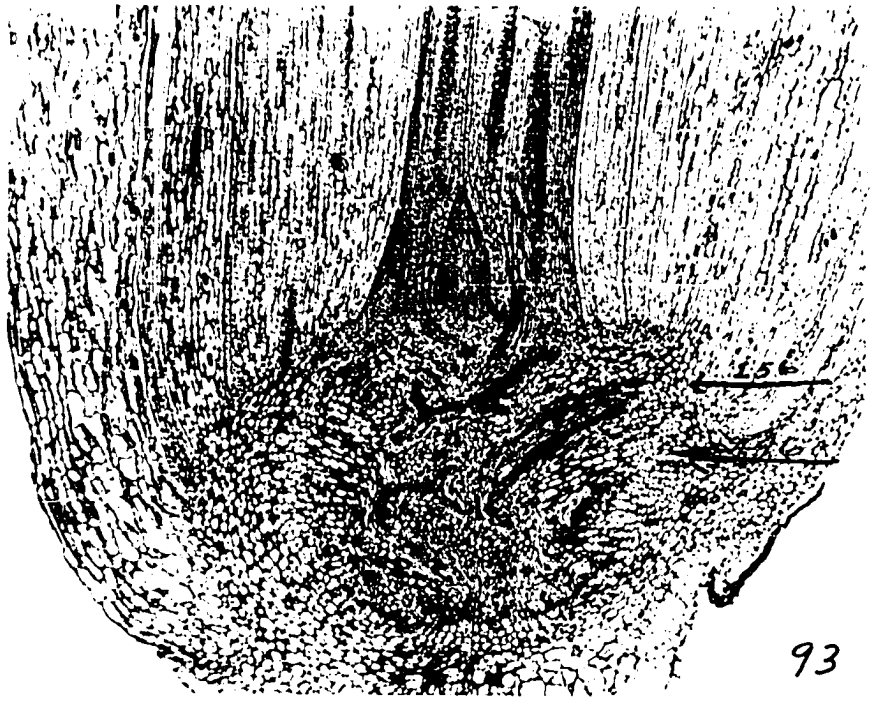
(fig. 8a, b, c) secondary growth occurs very near the tip.

As previously stated, the early enlargement of the axis is due to the addition of cells from the shoot apex and to the enlargement and division of the cells originally present in the embryonic axis. This early growth caused the stem to become obconical. However, as the seedling grows older, the activity of the primary thickening meristem becomes more intense at the sides of the axis, resulting in enlargement of the lower two-thirds of the stem (fig. 90). At the age of approximately 15 weeks the axis is more or less ellipsoidal (fig. 93). At a level of 256 μ below the base of the apical meristem (fig. 93), secondary growth is evident (fig. 94). The arrow on the left points to the secondary thickening meristem, inside of which young vascular bundles occur. On the right, the upper arrow indicates a vascular bundle at a later stage of development; the lower arrow points to the thickening ring. Figure 95 represents a median longisection of the axis of a 31-weeks' old plant. Compare the overall size of this axis with the one in figure 90.

The primary and secondary thickening meristems were studied next in small plants (fig. 7b, c) with stems approximately 6 mm wide. Stems at this age are usually more or less ovoid (fig. 8a) and show considerable secondary thickening. From its point of origin, the primary thickening meristem diverges laterally just below the leaves until it

Figure 93. A near-median longisection of the axis of a seedling 14 weeks old. The axis of plants of this age tends to be more or less oval in outline. Secondary growth is present in stems of this size at levels below the area of primary growth; the latter appears to extend down to a point about midway between the upper and lower limits of the stem. x39.

Figure 94. A transection 496 μ below the base of the apical meristem. At this level secondary growth is present, as indicated by the young vascular bundles just inside the secondary thickening meristem. The upper arrow on the right indicates a young vascular bundle, and the arrow below points to the thickening meristem. The arrow on the left also points to the thickening meristem. The second arrow from the top in figure 93 indicates the level at which this section was made. Note the interconnections between older and younger bundles. x128.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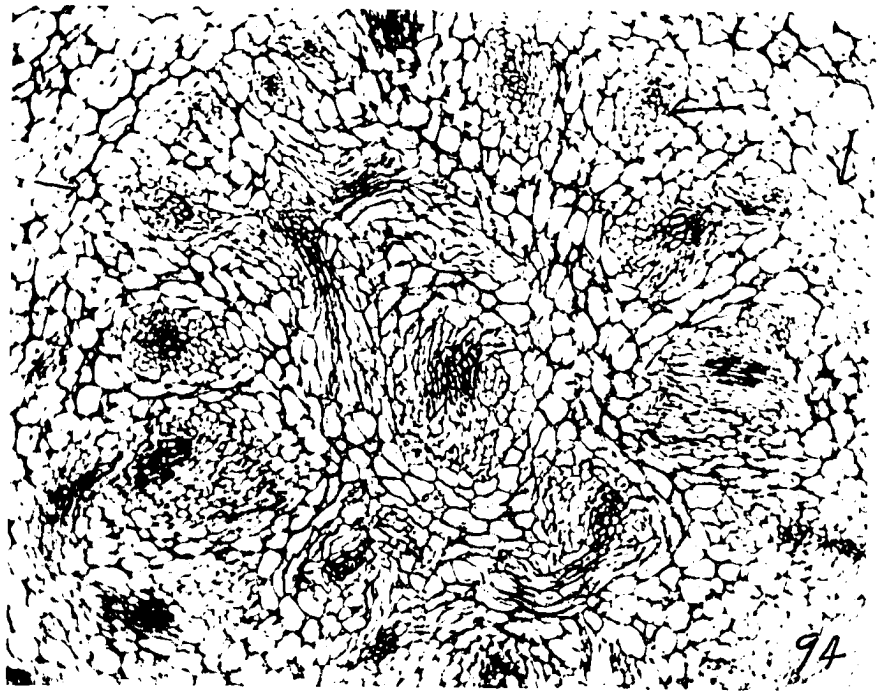
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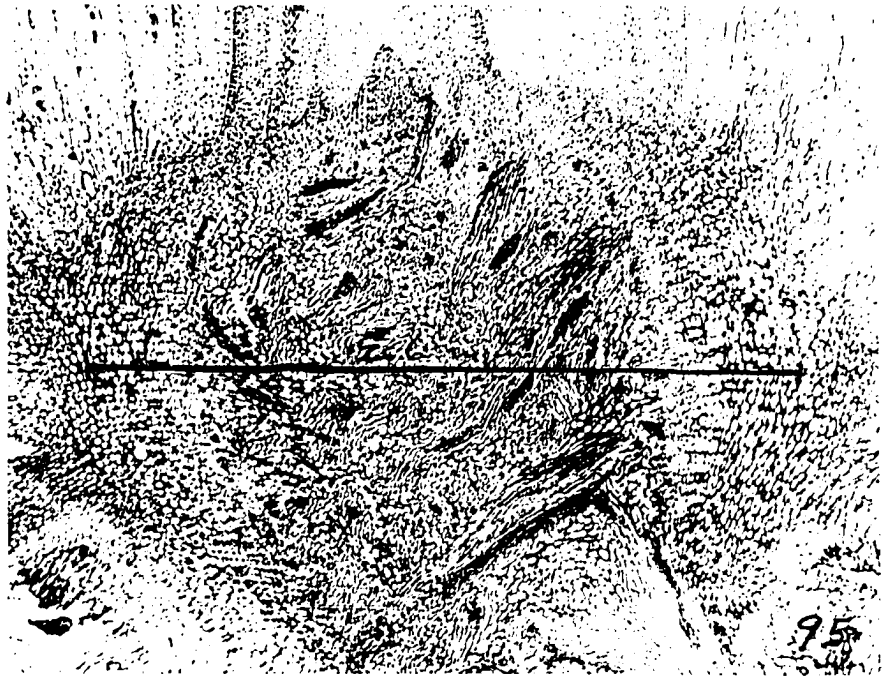
Figure 95. A near-median longisection of the axis of a seedling 31 weeks old. Note the young leaf traces in the primary part of the axis above, and the large, older traces of the first leaves below. Compare the size of this axis with that of a seedling 8 weeks old (fig. 90). x39.

Figure 96. A transection of a stem 7 mm in diameter. This stem came from a plant about the size of the one in figure 7c. On the outside of the thickening meristem the derivatives often divide radially, and then gradually enlarge and become a part of the cortex. Sometimes, however, they undergo a second radial division before enlarging, as indicated by the upper arrow. On the inside of the thickening meristem the radial arrangement of the cells is more or less maintained, especially between vascular bundles. Files of cells facing a vascular bundle almost invariably divide as indicated by the lower arrow. x150.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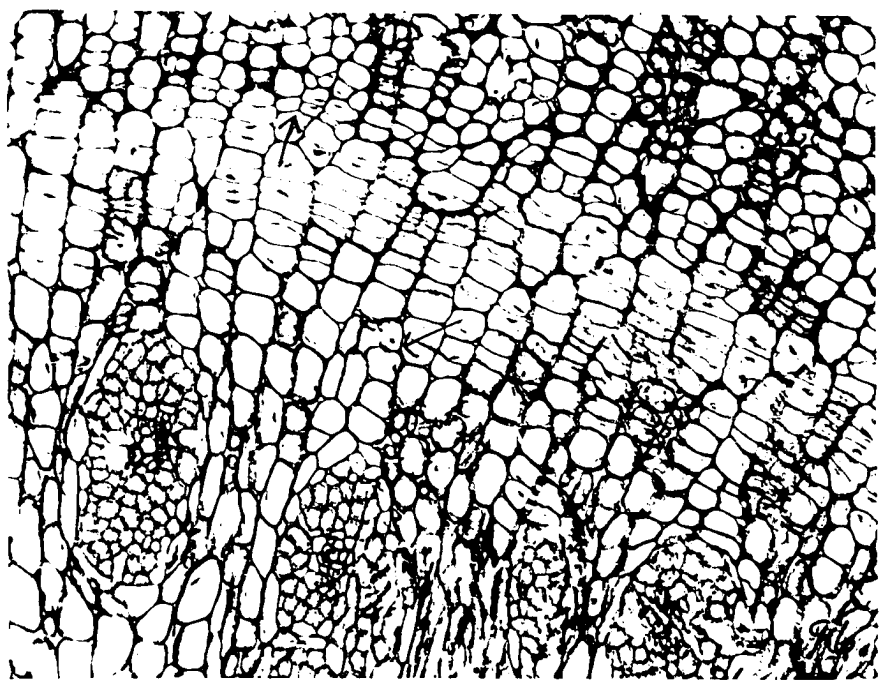


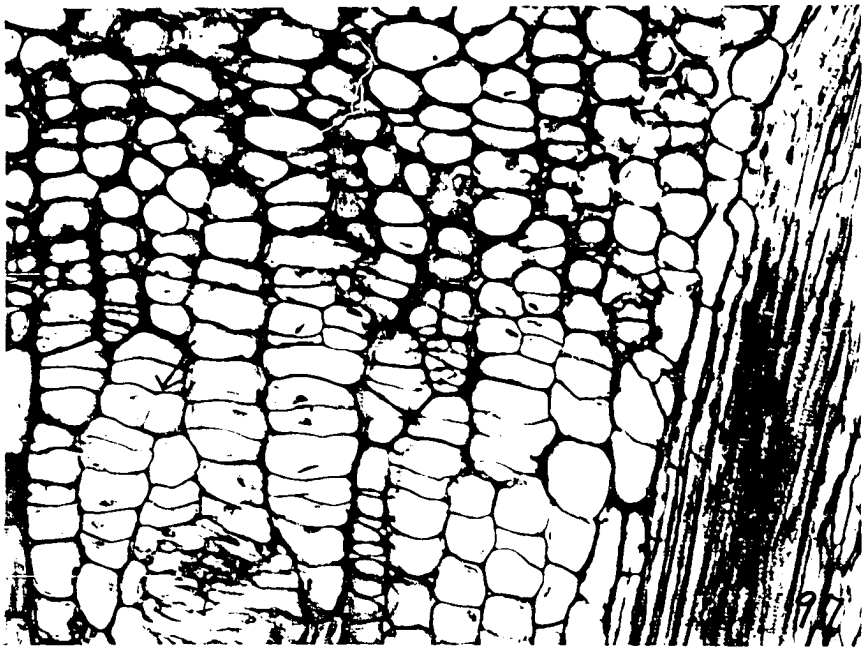
reaches the outer part of the stem tip, at which point it extends downward, parallel to the axis. Secondary growth appears to begin at about the point where the meristem takes a downward course. Transections at or somewhat below this point appear as the one in figure 96. The cells of the secondary thickening meristem (fig. 96) divide tangentially. Its outer derivatives divide radially, enlarge, and become parenchyma cells of the cortex. Cells of the cortex (figs. 96, 97) are at first in radial rows, but as they undergo divisions in various planes and enlarge they soon become irregularly arranged (fig. 97). Inside the thickening meristem, the radial arrangement of the cells is more persistent, especially between the vascular bundles. The mature secondary vascular bundles (fig. 98) appear elliptical in transection. They consist of a small inner core of phloem surrounded by a much larger amount of xylem, which in turn is surrounded by a sheath of parenchyma. They are therefore amphivasal. In figures 96 and 98 the secondary vascular bundles tend to be arranged in radial rows.

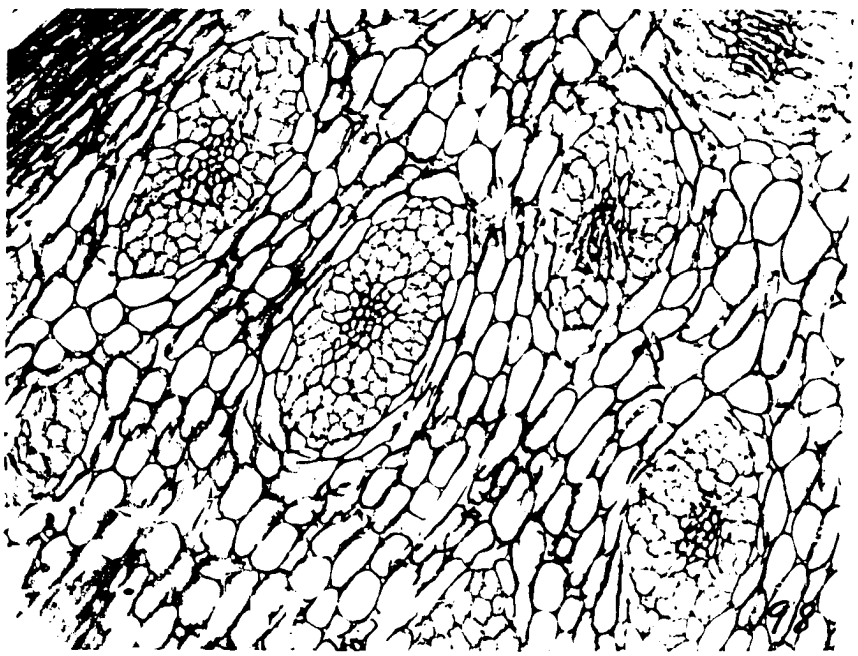
As new vascular bundles originate within the secondary thickening meristem, they anastomose with the older leaf traces. A large leaf trace is shown in figure 97, at the right. Figure 94 shows the connections of several young vascular bundles with older, more centrally located ones.

Secondary growth in the stems of mature plants (fig. 16) is extensive. Such stems are strong and woody and may be

Figure 97. A transection of a stem 7 mm in diameter. Divisions in the thickening meristem are generally tangential, but occasionally a radial one occurs, as indicated by the arrow. Note the large leaf trace. x228.

Figure 98. A transection of a stem 7 mm in diameter. This section is inside the thickening meristem, and shows the mature vascular bundles in the transection. Note the sheath of parenchyma which surround the xylem; the phloem is in the center. The bundles are amphivasal. x150.





several inches in diameter at their bases. It is sometimes possible, if the plant is not too old, to identify a small knob at the base of the stem (fig. 8f) which represents what remains of the early axis.

CHAPTER V

DISCUSSION

The development of the shoot apex in Dasylirion has been traced from a small cluster of cells in the embryo to a large dome-like meristem in the adult plant.

The second tunica layer, taking its origin from upper corpus initials, appeared to be well established after the initiation of the second leaf. Kasapligil (1951) states that in Umbellularia a well-established T-2 appears when the seedling is 15 months old and 3.5 cm. high. In Laurus a second tunica is well established when the seedling is 3 months old. Ball (1941) reported that in Phoenix canariensis the apices of young plants do not exhibit the zonation characteristic of the apices of adult plants until they have attained a shoot diameter of 5 cm. Reeve (1948a) states that in Pisum stratification develops during growth of the embryo. The apex of the adult is organized in the same manner as that of the fully developed embryo. Hsü (1944) reported that in Sinocalamus the lateral shoot apex differs at first from that of the main shoot, but later in life becomes similar to it.

In seedlings of Dasylirion centrally situated cells

of T-2 often undergo periclinal divisions during leaf initiation and development. A similar situation appears to exist in Salix (Reeve, 1948a). Reeve reports that the second layer of the tunica periodically divides periclinally, contributing cells to the corpus and forming a new second layer. According to Reeve "fluctuations in stratification reflect a dynamic relationship between apical organization and histogenesis, and constitute an evolved process of maintaining the primordial meristem." Reeve does not believe that the occurrence of periclinal divisions in the tunica makes the tunica-corpus concept unacceptable. Boke (1940) pointed out that irregularities in the tunica and corpus "emphasize the fact that these are interdependent zones of growth, and that, therefore, the concept of "tunica-corpus" should be dynamic in character." In Drimys lanceolata (Gifford, 1950) periclinal divisions have been observed in "central medianly positioned T-2 cells during minimal-area phase." They may also occur in the flank-cells of T-2 in Vinca rosea (Cross and Johnson, 1941). Boke (1947) reported them in cells of T-2 near the tip of the apex.

If the tunica-corpus concept were applied in its strictest sense to the seedling apex of Dasylirion, the tunica would possess but 1 layer. In this investigation, however, a dynamic approach to the description of the shoot apex has been used, similar to the one expressed by Reeve in 1948.

Boke (1941) states that "the tunica-corpus theory, championed by Foster (1939), has found wide usage in describing the anatomy of shoot apices in angiosperms." Comprehensive reviews of the shoot apex are numerous (Foster, 1939b, 1941a; Sifton, 1944; Philipson, 1949; Johnson, 1951; Popham, 1951; Esau, 1953). Gifford (1954) has reviewed "the more recent literature pertaining to the shoot apices of seed plants particularly the angiosperms."

In the development of the shoot apex of Dasylirion from the small cluster of cells in the embryo to the dome-like apex of the mature plant, there is a gradual increase in size and in the number of tunica layers. Growth in height occurs soon after the establishment of T-2. This is followed by an increase in width, which is greatest in a plane at right angles to the cotyledonary slit. After this, this apex increases for a time mostly in the plane of the cotyledonary slit. Later, the meristem undergoes a broadening and flattening until the apex characteristic of the mature plant is attained.

According to Rösler (1928) the architecture of the shoot apex in Triticum changes in the course of development. The structure of the apex in Avena undergoes a gradual change from the beginning of germination (Kliem, 1937). Whaley (1939) reported that in Lycopersicum the volume of the apical meristem increases as growth occurs, and reaches a maximum late in the grand period of growth. After this, it falls

off sharply to a relatively constant size. A somewhat similar situation appears to exist in Phoenix, as reported by Ball (1941). In Phoenix, the shoot apex achieves its maximum volume when the shoot diameter reaches 23 cm. No further increase in volume occurs while the shoot diameter grows to the normal of 45 cm. Actually, "there is a slight decrease until the volume characteristic of the mature palm is attained." Popham and Chan (1950) concluded from their study of Chrysanthemum that "there is apparently no correlation between the number of mantle layers and (1) the volume, height, or diameter of the shoot apex (2) presence of a zone of cambial-like cells (3) the volume of the central mother cell region (4) stage of the plastochron (5) the position of the bud on the stem. Rouffa and Gunckel (1951a) made a comparative study of vegetative shoot apices in the Rosaceae. They found that there was no correlation between size of the apex and the number of tunica layers. Sizes of the apices varied widely between species and between active shoots (terminal) and perennating buds (terminal and axillary). In Umbellularia (Kasapligil, 1951) the shoot apex at maximum area during the dormant stage has a width which varies from 112 μ to 120 μ. In the elongating shoot the width ranges from 147 μ to 222 μ. Abbe and Phinney (1951) studied the growth of the shoot apex in maize. They found that the shoot apex increases in general average size from plastochron to plastochron, and that "apparently a linear relationship

exists between the dimensions of the shoot apex in successive stages. Abbe, Phinney and Baer (1951) state that "the average rate of growth per stage, both for area and cell number, accelerates exponentially through successive stages." Abbe, Randolph, and Einset (1941) found that the shoot apex in diploid maize increases in circumference as it passes thru developmental stages. They found that this increase was due to an increase in cell number, while cell size and nuclear size remained essentially uniform.

Shoot apices of vascular plants vary in diameter from less than 80 μ in certain grasses (Foster, 1941) to about 3000 in certain cycads (Foster, 1941). Boke (1952) reported that the shoot apex of Mammillaria heyderi "reaches a maximum diameter of about 1500 μ, the largest yet described for any flowering plant." In Dasylirion, the diameter of the adult shoot apex at maximum area appears to vary from 392 to 488. In general it may appear that a large apex is associated with a relatively large primary axis. This is seemingly the case in the cycads (Foster, 1941) and in certain cacti (Boke, 1951, 1952, 1953). On the other hand, Ball (1941) has shown that certain palms which have a very large primary axis may have a shoot apex only 300 μ in diameter. Opuntia cylindrica has a shoot apex larger than that of the palm, yet its axis is less than an inch and a half in diameter. Pereskia cubensis (Boke, 1954) with a shoot apex nearly 300 μ in diameter has a primary axis no larger than many woody angiosperms which

have shoot apices only half as large.

The five zones in the shoot apex of Dasylirion are established when the emergent seedling is about 12 mm long, but they do not reach maximum development until the plant is fully grown. Millington and Gunckel (1950) point out that "it is only recently that zones supplemental to tunica-corpus have been recognized in the apices of angiosperms (Boke, 1941; Ball, 1941; Majumdar, 1942; Reeve, 1942, 1948; Hsü, 1944; Philipson, 1947, 1949)."

As the apex in Dasylirion increases in size, periclinal divisions in the outer corpus become fewer, resulting in the gradual establishment of additional tunica layers. In the fully developed meristem these layers vary in number from 6 to 7, which is more than has been reported for any other monocotyledon so far.

One periclinal division was observed in T-1. This was found in the apex of a stem 10 mm wide and 10 mm long. Such divisions have also been reported in other monocotyledons (Maize, Sharman, 1940; Agropyron, Sharman, 1943; Chlorogalum, Sterling, 1944; Saccharum, Thielke, 1951). Gifford (1954) points out that the 1-layered tunica common in many monocotyledons, correlated with the active participation of this layer in leaf development and occasional periclinal divisions in this layer at the shoot tip, invites comparison with gymnosperm shoot apices.

In Dasylirion the shoot apex of the seedling has a

1-layered tunica at first, but a second tunica layer is established early in development. After additional layers are formed the number characteristic of a plant at any particular size appears to vary. Gifford (1954) in discussing the work of Schnabel (1941) on Silene maritima points out that "only by extensive studies can the minimal and maximal limits of stratification be obtained for any given species." Schnabel concluded that Silene has a 3-layered tunica, but he showed that periclinal divisions may occur, even in T-2. The frequency of periclinals in T-2 was low but "illustrated that the autonomy of the two subsurface layers is not absolute (Gifford, 1954)." Gifford points out further that it has been found that "considerable variation exists as to the stability of the tunica layers above the youngest definable leaf (Foster, 1935; Boke, 1940; Reeve, 1948a; Rouffa and Gunckel, 1951; Millington and Gunckel, 1950; Gifford, 1950)."

Popham and Chan (1950) report a zone of cambium-like cells in Chrysanthemum, "extending from the surface, through the central portion of the apex, to the surface of the shoot tip." This type of zonation has been reported in Bellis perennis, Opuntia cylindrica, and Trichocereus spachianus. It has also been reported in a few monocotyledons and several gymnosperms including most, if not all, cycads. This cup-shaped zone is also present in the shoot apex of Dasylirion. It appears during the development of the early stages of zonation and is of common occurrence in the apices of large

plants. Gifford (1954) reports that a pattern similar to that in Chrysanthemum also occurs in Drimys winteri var. chilensis and Drimys lanceolata.

The cells of the corpus zone in Dasylirion in general stain lightly in contrast to the cells of the flank meristem. Gifford (1954) points out that there are several reported instances of this (Ball, 1941; Boke, 1941; Majumdar, 1942, Hsü, 1944; Philipson, 1947, 1949; Millington and Gunckel, 1950; Gifford, 1950; Rouffa and Gunckel, 1951a; Kasaphigil, 1951).

Leaf initiation in Dasylirion begins with periclinal divisions deep in the flank meristem. Once these divisions have begun they gradually spread to the outer layers but usually stop at the second. Occasionally the first layer is involved.

Leaf initiation in other monocotyledons appears to vary considerably. In Typha angustifolia and Heteranthera dubia (Schalscha-Ehrenfeld, 1940) leaf initiation begins with periclinal divisions in the corpus derivatives. In Tradescantia albiflora (Rüdiger, 1940) periclinal divisions occur only in the second tunica layer in the formation of the primordium. In Iris germanica (Rüdiger, 1940) leaf initiation begins with divisions in the outer layer of the corpus. Later divisions occur in T-2. In Elodea (Stant, 1952) anticlinal divisions occur first in the 1-layered tunica, and then periclinals occur in the outer corpus. Anticlinal divisions

in the tunica precede periclinals in the corpus in Convallaria majalis, Carex hordeistichos, Luzula sylvatica and Narcissus pseudo-narcissus (Stant, 1952).

Thielke (1951) reported that in grasses with 1 tunica layer the leaves develop either from the dermatogen or from the dermatogen plus corpus derivatives. In cases where 2 tunica layers are present both take part. With Saccharum, which has no stable tunica, the leaves arise from the corpus.

In Vanilla planifolia (Rüdiger, 1940), which has a 3-layered tunica, leaf initiation begins with periclinals in the third layer. Divisions later spread to the second tunica layer. In Sinocalamus (Hsü, 1944) initiation of the cataphyll and foliage leaf begins with periclinals in T-1. Later, divisions in T-2 occur. In cases where there are 3 tunica layers, the third layer does not take part.

Sharman (1940) points out that the outer layer in monocotyledons is frequently concerned in the initiation of leaves and often contributes considerably to their inner tissues. Sharman found that "in more than 20 species of grasses periclinal dermatogen divisions play their part in leaf primordium initiation." However, the outer layer appears to maintain itself in Typha, Potamogeton, Heteranthera, Trodescantia, Iris, Aloe, Vanilla, Tulip, and usually in Dasylirion.

The leaf primordium of Dasylirion has distinct

abaxial and adaxial surfaces and thus is described as being "bifacial." This is true also in the Gramineae, Amaryllidaceae, Liliaceae and certain other families (Roth, 1949). Unifacial apices occur in certain other monocotyledons (Knoll, 1948; Roth, 1949; Thielke, 1948). In the development of a unifacial primordium growth in the first center of activity is interrupted and another growth center originates abaxially to the first, so that the leaf which develops consists only of the abaxial side.

Esau (1953) discusses the complexity of structure and development of monocotyledonous leaves and points out that "it is difficult to draw a parallel between the parts of these leaves and those of dicotyledenous leaves." She states further that "some workers suggest that the monocotyledenous leaf has no counterpart of the lamina of the dicotyledenous leaves, that it is a phyllome lacking a lamina (Arber, 1950)."

Gifford (1954) points out that "in certain reported instances (Cross and Johnson, 1941; Schnabel, 1941; Reeve, 1942, 1948a; Gifford, 1950) a more stratified appearance of the shoot apex (at maximal-area phase of the shoot apex) is reported to be correlated with the initiation of a leaf primordium." In Dasylirion the apices of seedlings appear to exhibit maximum stratification at maximum area, which is just before leaf initiation. Periclinal divisions occur regularly in centrally located cells of T-2, especially in

young seedlings. In older seedlings they occur less regularly. In medium-sized plants maximum stratification is also present at maximum area and irregularities in tunica layers occur at leaf initiation. In adult plants however leaf initiation causes little change in the stratification of the apex, and periclinal divisions in the lower tunica layers are few.

The primary thickening meristem in Dasylirion originates when periclinal divisions occur in the derivatives of zone 5. This occurs when the seedling is approximately 2 weeks old. It appears likely that the meristem develops at lower levels only after the zone of periclinally dividing cells in the meristematic parenchyma outside the stele becomes more or less continuous, which occurs when the emergent seedling is about 12 mm long.

The primary thickening meristem of Dasylirion is of the storied type. In a tangential view the arrangement of the cambial cells in a storied meristem is such that "the fusiform initials occur in horizontal tiers with the ends of the cells of one tier appearing at approximately the same level (Esau, 1953)." In the modern concept of a storied cambium, a given cell undergoes a limited number of divisions after which it becomes inactive. Further divisions are inaugurated in neighboring cells and the process is repeated. Storied primary thickening meristems have been reported by various workers since Scott and Brebner (1893) stated that in Aristea corymbosa a storied meristem arose 30 mm back of the

shoot apex. Lindinger (1908) reported that a primary meristem of this type occurred just below the shoot apex of Aloe dichotoma, while Huasmann (1908) stated that in Nolina recurvata it was situated in the upper part of the axis. Carano (1910) merely states that it occurs near the shoot apex.

The thickening meristem was thought to be pericyclic in origin in Aristea corymbosa (Scott and Brebner, 1893), Aloe ferox (Chamberlain, 1921), and in Yucca (Barkley, 1924). In Dasylirion it appears that at lower levels the meristem originates in the young cortex. Esau (1953) points out that in gymnosperms and angiosperms "there is commonly no layer separating the cortex from the vascular tissue." Baranetzky (1894) concluded that the primary cortex as an embryologically autonomous tissue, does not occur in the monocotyledons.

The primary thickening meristem in Dasylirion goes through a definite developmental history, similar in certain respects to that in the palm, as described by Ball (1941). The meristem is not present in the embryo but develops in the seedling, where it assumes the shape of an inverted cone. In later seedling stages it tends to become almost flat. In the adult plant it is a concave region. These changes are associated with the formation of the apical depression.

Secondary growth in Dasylirion begins when young vascular bundles are formed inside the thickening meristem. This appears to occur about midway between the upper and lower extremities of the axis when the seedling is about 11 weeks

old. The primary thickening meristem thus intergrades with the secondary thickening meristem. This agrees with Lindinger's (1908) conclusions concerning Aloe dichotoma. He thought that the primary meristem in this plant was continuous with a secondary meristem further down, and that the two formed a meristem mantle from the tip to the base of the plant. He believed that the meristem producing primary thickening in monocotyledons is in no essential manner different from the secondary meristem found in some of them. Eckardt (1941) attempted to account for the origin of the secondary meristem. He concluded that the secondary meristem arises from the primary meristem. He states that there is no gap between primary and secondary growth in thickness, but that both types of growth arise by one and the same cambium which originates from the promeristem of the "Vegetationspunkt."

Various earlier workers also expressed doubts as to the existence of any real distinction between the two types of meristems (Schacht, 1852), Hausmann (1908), and Carano (1910).

Schoute (1902) was of the opinion that there were no satisfactory anatomical criteria on which to base a distinction between primary and secondary tissues. Such distinction as could be made, he believed should be done on physiological grounds. Primary tissues are those which are formed directly from the embryo or the apical meristems. Secondary tissues are formed after growth in length has ceased and bring about

an increase in conductive capacity.

The term cambium is used repeatedly in the literature on thickening in monocotyledons. Von Mohl (1858) stated that a cambium in the sense of that in the dicotyledons did not exist in the monocotyledons. Baranetzky (1897) called a cambium any tissue which produces radially arranged files of cells. Sanio (1863) believed that the presence of initials is the principal characteristic of a cambium. Schoute (1902) disagreed with Sanio's concept. Schoute states that "Ein Cambium ist eine ein oder mehrere Zellen breite Gewebezone, in der fast nur durch tangentiale Wände Teilungen stattfinden, in den meisten Fällen mit grosser Lebendigkeit. Von den so entstandenen Zellen erleiden die äusseren oder die inneren oder beide eine Ausbildung, wobei weitere Teilungen in diesen Zellen Aufhören." Helm (1936) defined a cambium on the basis of radial arrangement of cells.

The mature secondary vascular bundles in Dasylirion consist of a small inner core of phloem surrounded by a much larger amount of xylem, which in turn is surrounded by a sheath of parenchyma. They are therefore amphivasal. Sifton (1944) in reviewing the work of Scott and Brebner (1893), Hausmann (1908), Chamberlain (1921), and Cheadle (1937) states that "in secondary tissue, the development of each vascular bundle begins with longitudinal divisions in a single strand of cells." "One or two adjacent cells subsequently divide in a similar fashion, and a bundle results identical in appearance with the procambial strands of primary tissues."

CHAPTER VI

SUMMARY

Dasylirion leiophyllum is a xerophytic fibrous-leafed plant which occurs only in North America. Although considered to be a member of the Liliaceae in older classifications, it has recently been placed in the Agavaceae.

During the growth of Dasylirion from an embryo to a mature plant the shoot apex undergoes a definite developmental history. In the embryo it is a small cluster of cells at one side of the much larger primordium of the first leaf. In a seedling and a young adult it is asymmetrical, except near its base, appearing cone-like when viewed in the plane of the cotyledonary slit, but dome-like when viewed in a plane at right angles to the cotyledonary slit. In older plants the apex is symmetrical and appears in median longisection as a broad dome. In very large plants it appears to be about 63μ high and from 392 to 448 μ wide.

The five zones characteristic of the apical meristem are established by the time the emergent seedling is about 12 mm long. These include (1) a 1-layered tunica, (2) a corpus, (3) a flank meristem, (4) a rib meristem and (5) a zone below the flank meristem and lateral to the rib meristem. During

subsequent development the apex undergoes a gradual increase in size. Growth in height occurs soon after the establishment of T-2. This is followed by an increase in width, which is greatest in a plane at right angles to the cotyledonary slit. After this, the apex enlarges for a time mostly in the plane of the cotyledonary slit. Later, the apex undergoes a broadening and flattening which results in the establishment of the adult apical meristem.

As the apex increases in size, periclinal divisions in the outer corpus become fewer, resulting in the establishment of additional tunica layers. In the adult shoot apex these appear to vary from 6 to 7. Although periclinal divisions occur regularly in T-2 in young seedlings during leaf initiation and development, they appear to be less frequent as additional tunica layers are added. In the fully developed shoot apex they occur very infrequently.

Leaf initiation begins with periclinal divisions deep in the flank meristem. Once the divisions have begun, they spread outwardly, occasionally reaching the first layer. These divisions result first in the formation of a leaf-buttress, but soon they spread laterally. A primordium then arises as a protuberance on the foliar buttress and in seedlings, gradually encircles the apex somewhat like a collar. The primordium then continues development by a combination of apical and marginal growth, while intercalary growth at its base continues elongation. Procambium becomes visible in

both median longisection and transection when the primordium reaches 28 μ above the upper limit of the shoot apex. Apical and marginal growth soon cease, but elongation continues by means of intercalary growth. Growth inside the primordium then occurs by general cell division. Later, thickening of the leaf occurs by means of adaxial and abaxial meristems. Marginal growth occurs by means of initials which develop after additional procambial strands are formed.

The primary thickening meristem originates when periclinal divisions occur in the derivatives of zone 5. It probably develops at lower levels when the sporadic periclinal divisions in the meristematic parenchyma surrounding the stele become continuous. In a seedling 2 weeks old the meristem is visible in median longisection as a continuous zone on each side of the stele from points just below the shoot apex to the lower limit of the stem. As a result of divisions in the primary thickening meristem the upper part of the axis gradually expands upward and outward, while in the lower part the direction of growth is mostly outward.

Secondary growth appears to begin when young vascular bundles are formed within the thickening meristem about midway between the upper and lower limits of the axis. This has usually occurred by the time the seedling is 11 weeks old. Mature vascular bundles consist of a small inner core of phloem surrounded by a much larger amount of xylem, which in turn is surrounded by a sheath of parenchyma. They are, therefore, amphivasal.

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